Biochimica et Biophysica Acta 1834 (2013) 2564–2572

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbapap

The structural organization of the N-terminus domain of SopB, a virulence factor of *Salmonella*, depends on the nature of its protein partners

Pierre Roblin [a,b,1], Pierre Lebrun [c,1,2], Prakash Rucktooa [c], Frederique Dewitte [c], Zoe Lens [c], Véronique Receveur-Brechot [d], Vincent Raussens [e], Vincent Villeret [c], Coralie Bompard [c,*]

[a] *INRA Biopolymères, Interactions et Assemblages, Rue de la Geraudière, 44316 Nantes, France*
[b] *Synchrotron SOLEIL, L'orme des Merisiers, Saint Aubin, BP 48, 91192 Gif sur Yvette Cedex, France*
[c] *Interdisciplinary Research Institute, CNRS-USR 3078, Universite Lille Nord de France, Parc de la Haute Borne, 50 Avenue de Halley, 59658 Villeneuve d'Ascq, France*
[d] *Laboratory of Integrative Structural & Chemical Biology, Cancer Research Center of Marseille, INSERM U 1068/CNRS UMR 7258, Université Aix-Marseille/Institut Paoli Calmettes, 27 Boulevard Leï Roure-BP30059, 13273 Marseille Cedex 09, France*
[e] *Structure and Function of Biological Membranes, Université Libre de Bruxelles, Faculté des Sciences, Campus de la Plaine, CP206/02, Boulevard du Triomphe, 1050 Bruxelles, Belgium*

ARTICLE INFO

Article history:
Received 9 May 2013
Received in revised form 3 September 2013
Accepted 18 September 2013
Available online 26 September 2013

Keywords:
Type three secretion system
Small angle X-ray scattering
Intrinsically disordered protein
Bacterial virulence
Chaperone binding domain

ABSTRACT

The TTSS is used by *Salmonella* and many bacterial pathogens to inject virulence factors directly into the cytoplasm of target eukaryotic cells. Once translocated these so-called effector proteins hijack a vast array of crucial cellular functions to the benefit of the bacteria. In the bacterial cytoplasm, some effectors are stabilized and maintained in a secretion competent state by interaction with specific type III chaperones. In this work we studied the conformation of the Chaperone Binding Domain of the effector named *Salmonella* Outer protein B (SopB) alone and in complex with its cognate chaperone SigE by a combination of biochemical, biophysical and structural approaches. Our results show that the N-terminus part of SopB is mainly composed by α-helices and unfolded regions whose organization/stabilization depends on their interaction with the different partners. This suggests that the partially unfolded state of this N-terminal region, which confers the adaptability of the effector to bind very different partners during the infection cycle, allows the bacteria to modulate numerous host cells functions limiting the number of translocated effectors.

1. Introduction

Salmonella tiphimurium is a facultative intracellular pathogen that causes several diseases ranging from self-limiting enteritis to typhoid fever. Virulence factors are introduced in human cells by a specialized injection system, which is conserved in other gram negative pathogenic species. This system, named type three secretion system (TTSS), allows pathogenic gram-negative bacteria to deliver into the host cell a set of effector proteins (virulence factors) [1,2] in a contact-dependent manner. Substrates of TTSS are different enzymes that once inside the host cell can influence the signalling cascades [2,3]. These virulence factors allow the survival of pathogens under very specific conditions and represent attractive targets for antibacterial therapies.

Effector proteins can either be secreted by the bacteria to the culture supernatant or directly translocated into a eukaryotic host cell cytoplasm following induction by a host cell contact [4–8]. Most of these translocation processes require the presence in the bacterial cytosol of specific TTSS chaperones [9,10]. Proteins are thought to move into the TTSS apparatus in an unfolded state and their binding to specific cytoplasmic TSS chaperones is essential in the correct secretion [1].

The N-termini of the chaperone-dependent effector proteins harbour two different domains: the N-terminal secretion signal (NSS) and the Chaperone Binding Domain (CBD) [11–13]. The NSS is 20 amino acids long, and mediates secretion but is insufficient for translocation [14]. Whether the nature of this signal is due to the mRNA or the protein sequence of the effector is still a matter of discussion and to date no consensus sequence in the protein or the mRNA has been identified [10]. The translocation process of effectors relies on the binding of specific TTSS chaperone proteins on a small 50–100 amino acid domain next

Abbreviations: CBD, chaperone binding domain; TTSS, type three secretion system; SopB, Salmonella outer protein B; SAXS, small angle X-ray scattering; CD, circular dichroism; DSP, dithio-bis-(succinimidylpropionate); R_g, radius of gyration; R_h, hydrodynamic radius

* Corresponding author at: Interdisciplinary Research Institute, CNRS -USR 3078, Universite Lille Nord de France, Parc de la Haute Borne, 50 Avenue de Halley, 59658 Villeneuve d'Ascq, France. Tel.: +33 362 531 740.
E-mail addresses: pierre.roblin@synchrotron-soleil.fr (P. Roblin), pierre.lebrun@vub.ac.be (P. Lebrun), prakash.rucktooa@univ-lille1.fr (P. Rucktooa), frederique.dewitte@iri.univ-lille1.fr (F. Dewitte), zoe.lens@iri.univ-lille1.fr (Z. Lens), veronique.brechot@imm.cnrs.fr (V. Receveur-Brechot), vrauss@ulb.ac.be (V. Raussens), Vincent.villeret@iri.univ-lille1.fr (V. Villeret), coralie.bompard@iri.univ-lille1.fr (C. Bompard).
[1] These authors contributed equally to this work.
[2] Present address: Structural Biology, Brussels (SBB), Vlaams Instituut voor Biotechnologie (VIB) Vrije Universiteit Brussel (VUB), Pleinlaan 2, 1050 Brussels, Belgium.

1570-9639/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbapap.2013.09.014

to the NSS and called Chaperone Binding Domain (CBD) [11–13]. It has been proposed that the Chaperone/CBD complex might have multiple roles, such as stabilization of the effectors by the maintenance in a secretion competent state, or targeting of effectors to the TTSS apparatus and regulating the type III gene expression [9,15–18]. After secretion, the chaperones release their cognate substrates and remain within the bacterial cytoplasm. Recent results showed that the chaperone alone or in complex with the secreted effector is recognized by InvC an ATPase associated with the TTSS of *Salmonella* and this interaction plays a critical role in the substrate recognition by the TTSS machinery [19].

Class I chaperones are specific to one (class IA) or more (class IB) type III effectors [9]. Despite a lack of sequence similarity, TTSS chaperones share some features: a molecular mass around 15 kDa, an acidic isoelectric point, high specificity and affinity for their cognate effectors and they form highly stable dimers in solution [10]. Several structures of class IA chaperones [20,21] have been solved by X-ray crystallography and revealing that these proteins share a common structural fold and are structurally homologous despite a low sequence identity [20]. They all adopt a conserved heart-shaped-dimeric structure with a mixed α/β fold. This conserved fold binds and maintains the CBD of the cognate effector in an extended partially unfolded state [17].

To date several structures of effector CBDs in complex with their cognate chaperone have been solved. The SptP CBD (residue 35–139) surrounds both monomers of its chaperone SicP and is maintained mostly in an extended conformation [22]. The YopE CBD (residue 15–75) is bound to its specific chaperone SycE, similar to SptP, suggesting a universal binding mode between CBDs and chaperones even in the absence of a sequence conservation between these proteins [23]. The structure of the CBD of SipA (residues 48–264) has been solved alone and complexed with its chaperone InvB [24]. Unbound CBD is mainly composed by α-helices and presents a protrusion containing the first helix whereas the first 48 residues are disordered. Its interaction with InvB is different from what was observed for all known structure of CBD/chaperone complexes and occurs only with one protomer of the dimeric chaperone. Only a small part of the chain (residues 23–46) is maintained in an extended conformation and the global fold of the CBD is similar to the unbound CBD and behaves as a globular domain in contact with InvB by the first three helices. The presence of both disordered and globular domains are required for the interaction with the chaperone [24]. Structural alignment of these structures revealed a common structural β-motif both in the CBD of effectors of several species whose disruption leads to destabilization of chaperone–substrate complex and in chaperone structures [24]. The conserved effector β-strand interacts with the conserved first β-strand of the chaperone's β-sheet to form protein–protein interaction, namely β-sheet augmentation, that have been already described in many metabolic pathways [25].

The object of this study, SopB (also called SigD), a SPI-1-TTSS-translocated *Salmonella* effector, with a C-terminal phosphatidylinositol phosphatase domain that acts on host cell membrane phospholipids taking part to many cellular processes such as preventing apoptosis [26].

In the *Salmonella* cytoplasm, SopB is maintained in a secretion competent state by the interaction with its cognate chaperone SigE [27]. SigE is a class IA chaperone whose structure has been solved by X-ray crystallography and displays the conserved fold described for class IA chaperones [20,21]. SigE is essential for the stability, secretion and translocation of SopB. The SigE/SopB complex formation requires the dimeric state of SigE as well as the presence of conserved hydrophobic β motif described for class IA chaperone [21,24]. In this chaperone–effector complex the C-terminal active domain of SopB remains fully active [23].

In eukaryotic cells the biological phosphoinositide phosphatase activity of SopB is required in many processes affecting the bacterial entry and persistence into non-phagocytic cells [26,28–30]. After translocation, together with effectors SopE and SopE2, SopB interferes with cellular functions by activating the host Rho GTPase Cdc42 [31]. Cdc42 is an essential Rho GTPase that regulates cytoskeleton organization and membrane trafficking during cell motility, proliferation, and cytokinesis in eukaryotes [32]. Functional studies revealed that the N-terminal region of SopB binds specifically Cdc42 and that this interaction is important for translocation of SopB to the SCV [33,34]. The structure of the N-terminal domain of SopB (residues 29–181) in complex with Cdc42 has recently been solved [35]. Analysis of this structure shows no electronic density for residues from 29 to 45 and 171 to 181 suggesting that these fragments don't have a stable conformation in the complex. However the 46–171 domain of SopB behaves completely folded in a globular domain composed by an N-terminal unique β-strand (β1) followed by five α-helices (α1–5). The interaction with Cdc42 is mediated by β-augmentation of the β-sheet of Cdc42 by addition of β1 of SopB. This region of the complex closely resembles interactions between Cdc42 and eukaryotic effector proteins that contain a CRIB motif thus mimicking the eukaryotic regulators of small GTPases [35]. In the host cell, SopB is membrane-associated through residues 117–167 that are essential for membrane targeting [36] and undergoes several ubiquitinations after translocation that regulates its enzymatic activity as well as its intracellular localization. Most of these ubiquitination sites are localized at the N-terminus of the protein [36,37].

In this work we defined the size of the CDB of SopB, we analyzed its structure by SAXS, alone as well as in complex with its cognate chaperone SigE and we compared it with the known structure of SopB in complex with Cdc42. Our results show that CBD alone is mainly unfolded and probably undergoes induced folding by interaction with the different partners encountered during virulence cycle and that the ability of this region to interact with many different partners is probably promoted by its intrinsically disordered state.

2. Material & methods

2.1. Protein cloning, production and purification

The *Escherichia coli* strains XL1 and BL21(DE3) were used for construction of the plasmids and gene expression, respectively. SigE and all the constructs of SopB were amplified from *S. tiphimurium* genomic DNA and cloned in the petDuet vector (Novagen) which allows co-expression of multiple target genes in *E. coli* with restriction sites BlgII-XhoI and BamH1/NotI respectively inserting an N-terminal His6-tag on each SigD constructs. All versions of SopB, object of this study, were deleted in the first N-terminal 29 amino acids, as it was previously shown that the truncated form of SopB was still active and also able to bind SigE in a secretion competent complex [36]. Cells were grown in an LB broth supplemented with ampicillin (100 μg/ml) and induced for protein production with IPTG 0.5 mM at OD600 nm 0.6 for 3 h at 37 °C.

The SopB/SigE complex harvested cells were re-suspended in lysis buffer 1 PBS buffer added with 0.5 M NaCl, 5 μg/ml Desoxyribonuclease (DNase) and complete protease inhibitor cocktail EDTA free tablets (One tablet per Liter of culture), lysed by sonication and centrifuged at 12,000 *g* for 60 min at 4 °C. Soluble protein extract was purified using a Nickel chelating resin (GE Healthcare Life Sciences) followed by a gel filtration step using a HiLoad 16/60 Superdex 200 (GE Healthcare Life Sciences) pre-equilibrated in PBS, NaCl 150 mM, glycerol 10%, and Dithiothreitol (DTT) 2 mM.

The constructs of SopB described in Table 1 were co-expressed with SigE as described above. Complex formation with SigE was checked by co-purification trials on a Nickel chelatin resin (GE Healthcare life science).

CBD/SigE complex harvested cells were re-suspended in lysis buffer 2 (TRIS 100 mM pH 7.2) added with 5 μg/ml DNase and complete protease inhibitor cocktail tablets (Roche), lysed by sonication and centrifuged at 12,000 *g* for 60 min at 4 °C. Contrary to the SopB/SigE complex, the soluble protein extract couldn't bind to a Nickel chelating resin and