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Mechanistic characterization of sulfur transfer from cysteine desulfurase SufS to the iron–sulfur scaffold SufU in *Bacillus subtilis*

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ABSTRACT

Iron–sulfur cluster biosynthesis in Gram-positive bacteria is mediated by the SUF system. The transfer of sulfide from the cysteine desulfurase SufS to the scaffold protein SufU is one of the first steps within the assembly process. In this study, we analyzed the interaction between *Bacillus subtilis* SufS and its scaffold SufU. The activity of SufS represents a Ping-Pong mechanism leading to successive sulfur loading of the conserved cysteine residues in SufU. Cysteine 41 of SufU is shown to be essential for receiving sulfide from SufS, while cysteines 66 and 128 are needed for SufS/SufU interaction. In conclusion, we present the first step-by-step model for loading of the essential scaffold component SufU by its sulfur donor SufS.

Structured summary:
SufS and **SufU** bind by molecular sieving (View interaction)
SufS binds to **SufS** by molecular sieving (View interaction)
SufS and **SufU** redox react by enzymatic study (View Interaction 1, 2, 3, 4, 5)
SufU physically interacts with **SufS** by pull down (View Interaction 1, 2)

1. Introduction

Iron–sulfur clusters (Fe/S clusters) belong to the prevalent and most versatile group of cofactors in nature. Due to their structural variability and diversity of redox potentials they play important roles in many biochemical pathways such as respiration, iron storage, redox control or sulfur donation [1]. The biogenesis of Fe/S clusters in bacteria is dependent on complex machineries, such as the NIF [2], ISC [3] and SUF system [4]. These systems are widely distributed amongst bacterial species and higher organisms [5,6]. While Gram-negative bacteria like *Escherichia coli* possess the two Fe/S cluster biogenesis systems ISC and SUF, most Gram-positive bacteria such as *Bacillus* spp., *Enterococcus faecalis* or *Mycobacterium tuberculosis* only feature a SUF system for Fe/S cluster biosynthesis [5,7–9] (Fig. 1). In *Bacillus subtilis*, the single components of this system including the major scaffold protein SufU were shown to be essential [7].

During the assembly process, all Fe/S cluster biogenesis systems share common basic principles: The cluster is built upon a scaffold protein of the U- or A-type [10,11], on which different cluster types can be assembled and which can transfer the clusters subsequently to an array of target proteins [10–16]. In addition, SufB of the SUF system possibly represents a further type of scaffold proteins [17,18]. A further common feature is the presence of PLP-dependent cysteine desulfurases (NifS, IscS, SufSE in Gram-negatives or SufS in Gram-positives) employed for the incorporation of sulfur derived from cysteine [13,19,20]. These enzymes convert cysteine to alanine and transfer the sulfur to the corresponding scaffold proteins.

The interaction of cysteine desulfurase and scaffold protein for the ISC system has been part of previous studies [21,22]. The general suggested mechanism is that the sulfide is bound as a persulfide on an active site cysteine of the cysteine desulfurase, from which it is transferred to the conserved cysteines of the scaffold protein. It has been shown that this process involves the formation of a heterodisulfide complex between scaffold and cysteine desulfurase [21,23]. For the *E. coli* IscS/IscU interaction, it was shown that all three cysteine residues of IscU could be loaded by IscS, while a disulfide bond is formed between Cys328 of IscS and Cys63 of IscU [23]. In *Azotobacter vinelandii* it was found that Cys37 of IscU is important for the formation of the heterodisulfide complex with IscS [21].

In this study, we investigated the mechanism of sulfur transfer between SufS and SufU in *B. subtilis* as a model system for Fe/S cluster biogenesis in Gram-positive bacteria. By kinetic and

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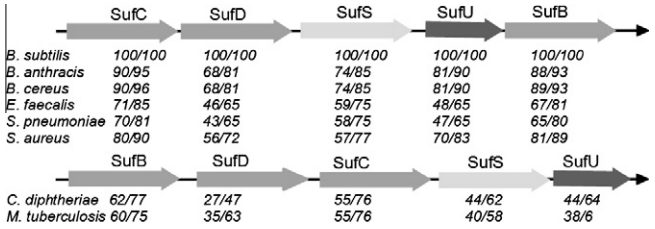

Fig. 1. The SUF gene cluster representing the common arrangement of the *suf* genes (medium grey: energy producing; light grey: cysteine desulfurase; dark grey: scaffold) in representative Gram-positive bacteria, indicating gene arrangements between Firmicutes and Actinobacteria. Homologies of the genes are indicated by identities/similarities of the amino acid sequences in % based on the corresponding *Bacillus subtilis* sequences.

inhibitory studies of SufS activity, we analyzed the influence of SufU and its single cysteine to alanine mutation variants on cysteine desulfuration. This led to a model proposing the single steps of sulfur transfer between the cysteine desulfurase and the major scaffold protein in the conserved Gram-positive SUF system.

2. Materials and methods

Materials and methods are described in the Supplementary data.

3. Results

3.1. Kinetics of sulfur transfer

As we have reported previously, the sulfide release activity of SufS increases up to 40-fold in the presence of *apo*-SufU [7]. In this study, we performed kinetic analyses of SufS activity under variation of both cysteine and *apo*-SufU substrates. The reactions were analyzed by the detection of the released amount of sulfide. Concentrations of cysteine were varied between 0 and 2 mM under constant SufU concentration, sufficient for maximum SufS activation (Fig. 2A), and varying SufU concentrations (0–10 μM) were also tested under non-limiting concentrations of cysteine (Fig. 2B). We found that the kinetics of SufS follow a Michaelis–Menten-like behavior under both cysteine and SufU variation. The determined kinetic parameters are listed in Table 1.

To determine the reaction mechanism, we analyzed the SufS activity with varied SufU and cysteine concentrations. We used 1, 2.5 and 5 μM SufU and varied cysteine from 10 to 50 μM, or alternatively from 10 to 1000 μM (Fig. 2C and Supplementary data Fig. S1). The parallel curves (within experimental error) of the double reciprocal plots indicate a Ping-Pong bi–bi reaction mechanism. The Ping-Pong type is supported by the fact that we could not observe cysteine bound to the PLP cofactor of SufS either directly after purification or after incubation with cysteine (Supplementary data Fig. S2), indicating a fast and stable loading of the catalytic SufS cysteine with the persulfide without requirement of SufU.

Kinetic data that were reported in parallel to this work by Selbach et al. [24] are in agreement with our observations.

3.2. SufS is able to reconstitute an Fe/S cluster on SufU

Since it is possible to build an Fe/S cluster under anaerobic conditions on SufU by using ferric ammonium citrate and cysteine in the presence of SufS (see Supplementary data Fig. S3) [7], we analyzed the amount of labile sulfide bound to the reconstituted *holo*-SufU after enzymatic reconstitution to determine the number of sulfide transfer cycles per SufU monomer until complete loading. After purification by size exclusion chromatography, an amount of 1.65 ± 0.05

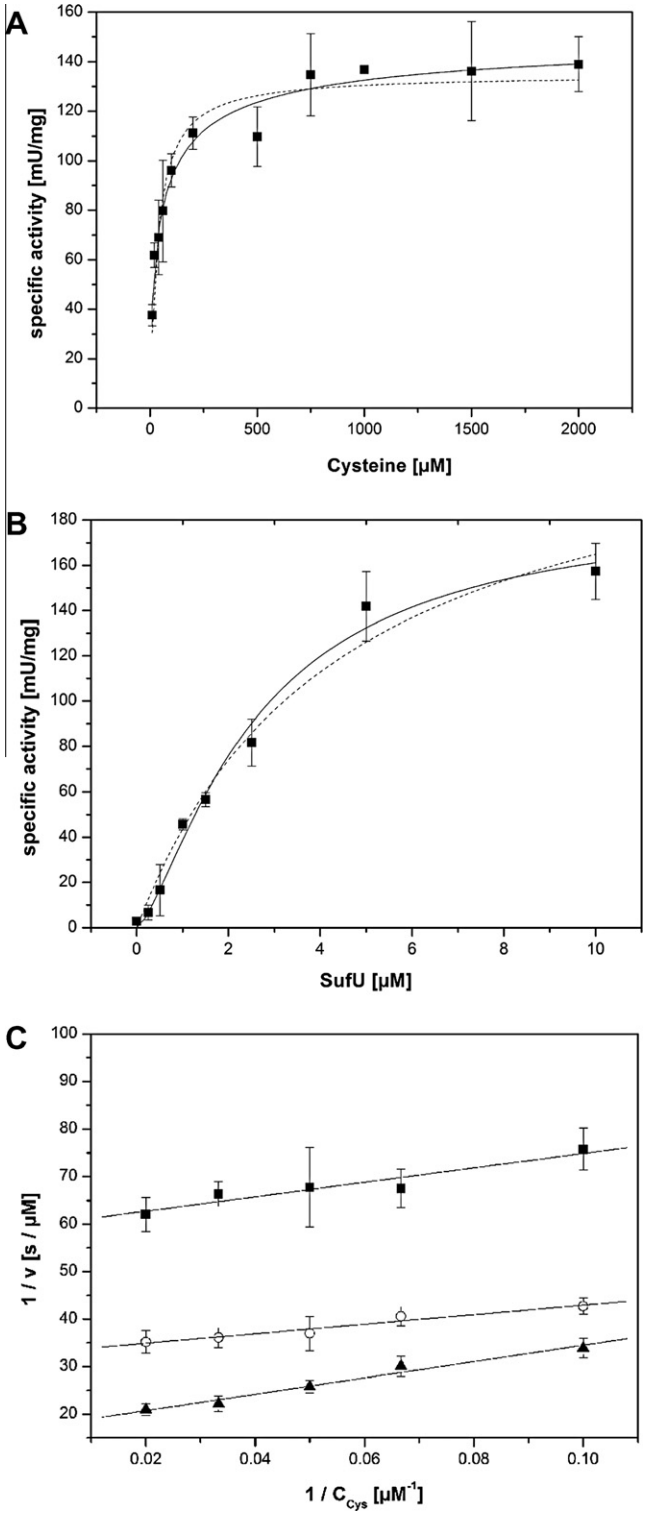

Fig. 2. SufS activity dependent on varied cysteine concentration (A) and SufU concentration (B). The reaction mixture contained 0.5 μM SufS, 10 μM SufU and 10–2000 μM cysteine (A), or 0.5 μM SufS, 0–10 μM SufU and 2 mM cysteine (B). Shown are the kinetic fits after Hill (solid line) and Michaelis–Menten (dashed line). (C) Double reciprocal plot of the reaction velocity of SufS with different SufU concentrations (1 μM ■, 2.5 μM ○, 5 μM ▲ SufU) against the cysteine concentration (10–50 μM) and the corresponding linear fits.

labile sulfide per SufU monomer was detected, indicating an average of two sulfide transfers until complete Fe/S cluster formation in vitro. Exact determination of the cluster type was not possible, since EPR and Mössbauer spectroscopy were not possible [7].