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Interaction between Sox proteins of two physiologically distinct bacteria and a new protein involved in thiosulfate oxidation

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ABSTRACT

Organisms using the thiosulfate-oxidizing Sox enzyme system fall into two groups: group 1 forms sulfur globules as intermediates (*Allochromatium vinosum*), group 2 does not (*Paracoccus pantotrophus*). While several components of their Sox systems are quite similar, i.e. the proteins SoxXA, SoxYZ and SoxB, they differ by Sox(CD)$_2$ which is absent in sulfur globule-forming organisms. Still, the respective enzymes are partly exchangeable in vitro: *P. pantotrophus* Sox enzymes work productively with *A. vinosum* SoxYZ whereas *A. vinosum* SoxB does not cooperate with the *P. pantotrophus* enzymes. Furthermore, *A. vinosum* SoxL, a rhodanese-like protein encoded immediately downstream of *soxXAK*, appears to play an important role in recycling SoxYZ as it increases thiosulfate depletion velocity in vitro without increasing the electron yield.

1. Introduction

Thiosulfate oxidation is conducted by a large number of photo- and chemotrophic sulfur oxidizing bacteria. In many of these organisms, the sulfur oxidizing (Sox) multienzyme complex is involved in the oxidation of thiosulfate to sulfate. Among these organisms, some store sulfur globules as intermediates (e.g. the purple sulfur bacterium *Allochromatium vinosum*) whereas others do not form sulfur deposits (e.g. *Paracoccus pantotrophus*) [1].

In *A. vinosum, sox* genes were identified in two clusters (ORFa-*soxBXA*ORF9(now *soxK*)*rhd*(now *soxL*)ORFbORFc and ORFd*soxYZ*-ORFeORFf), with *soxBXA* and *soxYZ* being indispensible for thiosulfate oxidation [2]. The latter genes code for the three periplasmic proteins SoxXA, SoxB and SoxYZ. SoxXA from *A. vinosum* is a heterodimeric heme protein with one CXXCH heme *c*-binding motif in each of the two subunits whereas the counterpart from *P. pantotrophus* is a tri-heme protein [3]. Furthermore, SoxXA of *P. pantotrophus* acts as heterodimer whereas recently it has been suggested that SoxXA of *A. vinosum* is a trimer additionally binding SoxK, the product of the gene immediately downstream of *soxA* [4]. The monomeric SoxB of *P. pantotrophus* contains a dimanganese cluster [5]. The cluster-coordinating aspartate residues are conserved in the *A. vinosum* protein. SoxYZ is a heterodimer that does not contain any cofactor or metal. In *P. pantotrophus* 15 open reading frames were identified in one *sox* gene cluster. It is organized in three transcriptional units (*soxRS*, *soxVW*, *soxXYZABCDEFGH*; [6–8]). These encode for the three core Sox enzymes SoxXA, SoxB and SoxYZ as well as a fourth enzyme Sox(CD)$_2$ and other presumably regulatory proteins. Sox(CD)$_2$ is an $\alpha_2\beta_2$, heterotetrameric molybdoprotein cytochrome *c* complex which is not present in *A. vinosum* [2,9].

The currently proposed modes of action for the two different Sox multienzyme systems are shown in Fig. 1. In both cases, thiosulfate oxidation is initiated by the oxidative coupling of thiosulfate to a conserved cysteine residue close to the carboxy-terminus of SoxY mediated by SoxXA, thereby releasing two electrons. In the next step SoxB hydrolytically releases one molecule of sulfate to yield a SoxY-persulfide. The subsequent reaction has been proposed to differ in the two organisms: in *P. pantotrophus* the sulfane dehydrogenase Sox(CD)$_2$ oxidizes the sulfane sulfur of the SoxY-persulfide to SoxY-thiosulfonate with the concomitant release of six electrons, SoxB again hydrolytically cleaves off the terminal sulfone moiety as sulfate and releases SoxYZ for a new reaction cycle. In *A. vinosum* Sox(CD)$_2$ is not present but SoxY still has to be regenerated for the next cycle of thiosulfate binding. Therefore, the sulfane moiety was proposed to be transferred to finally form sulfur globules [2].

Abbreviations: DTT, dithiothreitol; Sox, sulfur oxidizing

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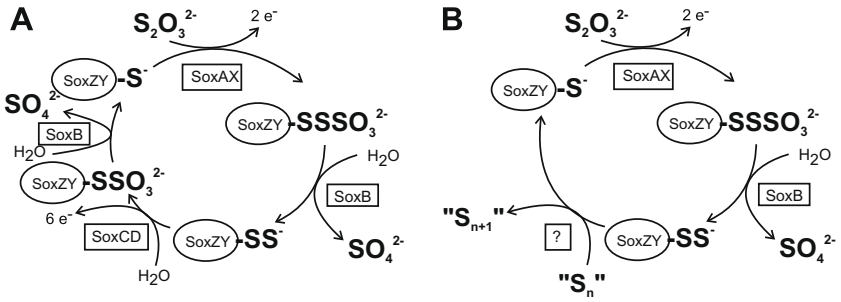

Fig. 1. Model of Sox-mediated thiosulfate oxidation in *P. pantotrophus* (left) and *A. vinosum* (right) (modified after [21] with permission).

The ability of SoxY to carry sulfur moieties at its C-terminus has been experimentally documented [10,11].

We here report the heterologous production of *A. vinosum* Sox proteins in *Escherichia coli* and the reconstitution of the Sox enzyme system in vitro. We demonstrate that the core Sox proteins SoxB and SoxYZ are interchangeable in almost all tested combinations of the enzyme systems of the sulfur globule-forming phototroph *A. vinosum* and the chemotroph *P. pantotrophus*, an organism that oxidizes thiosulfate without the formation of intermediates. We furthermore present evidence that the sulfane dehydrogenase Sox(CD)$_2$ of *P. pantotrophus* functions with the Sox proteins of *A. vinosum* in vitro. The rhodanese-like SoxL, a protein not present in *P. pantotrophus*, is shown to be a likely candidate for recycling of SoxYZ in *A. vinosum*.

2. Materials and methods

2.1. Production and purification of Sox proteins

P. pantotrophus Sox proteins were purified as described by Quentmeier and Friedrich [10]. The *A. vinosum soxYZ*, *soxB*, and *soxL* (formerly *rhd* [2]) genes (GenBank accession numbers DQ441405 and DQ441406) were amplified by PCR using the GC-RICH PCR system (Roche Diagnostics, Germany). The genes *soxYZ* were cloned into pET22b (Invitrogen, Karlsruhe) without the signal peptide-encoding sequence using *Nde*I and *Hin*dIII such that a His-tag was fused to the C-terminus of SoxZ. SoxYZ was then overproduced in the *E. coli* BL21(DE3) (Novagen, Madison) cytoplasm and purified using the His-tag (Qiagen, Hilden). The protein was stored at −70 °C in 25 mM K_2HPO_4/KH_2PO_4 buffer containing 1 mM $MgSO_4$ and 0.1 mM $Na_2S_2O_3$ (pH 6.5). *A. vinosum soxB* was cloned without the signal peptide-coding sequence into pPR-IBA1 (IBA, Göttingen) using *Xho*I and *Pst*I. The resulting plasmid enabled cytoplasmic overproduction of C-terminally Strep-tagged SoxB in *E. coli* BL21(DE3) which was then purified by affinity chromatography according to the manufacturer's instructions (IBA, Göttingen). The recombinant protein was stored at −70 °C in 10 mM Tris, 1 mM $MgSO_4$, 0.1 mM $Na_2S_2O_3$, 10% (v/v) glycerol (pH 7.5). The *soxL* gene was amplified without its intrinsic signal peptide and cloned into pET15b or pET22b using *Nde*I/*Bam*HI resulting in N-terminally or C-terminally His-tagged proteins directed to the *E. coli* cytoplasm. In addition, *soxL* was cloned into pET22b such that it was fused to a carboxy-terminal His-tag and joined N-terminally to the *pelB* leader sequence directing the protein to the *E. coli* periplasm. The proteins were overproduced in *E. coli* BL21(DE3), purified via affinity chromatography and stored at −70 °C in 10 mM Tris buffer containing 10% (v/v) glycerol.

2.2. Sox enzyme assays

All enzyme activity assays were performed at 30 °C in a UV–VIS-NIR recording spectrophotometer (UV-3100, Shimadzu). The reactions were performed in a total volume of 700 μL with 70 μM horse heart cytochrome *c* and 100 μM thiosulfate. The reduction was recorded as increase of absorption at 550 nm. One unit enzyme activity was defined as 1 μmol cytochrome *c* reduced per min and mL. Activities were calculated using a molar extinction coefficient ε for horse heart cytochrome *c* of 21.1 cm^2 μmol^{-1} [12]. The reaction was started by addition of SoxYZ which was activated prior to enzyme assays. For the activation, 50 μM SoxYZ solutions were incubated with 1.3 mM Na_2S for 20 min at 30 °C, and then washed three times with VivaSpin500 devices (Sartorius, Göttingen) to eliminate Na_2S [13]. Assays with *A. vinosum* SoxYZ and *P. pantotrophus* Sox enzymes (SoxXA, SoxB, Sox(CD)$_2$) were performed in 50 mM bis-Tris buffer (pH 6.0). Assays with *A. vinosum* SoxB, *A. vinosum* SoxYZ and *P. pantotrophus* SoxXA and Sox(CD)$_2$ were performed in 50 mM MOPS buffer (pH 7.2). Standard enzyme concentrations were 0.5 μM for *P. pantotrophus* Sox enzymes, 2.5 μM for *A. vinosum* SoxYZ and 0.05 μM for *A. vinosum* SoxB. Whenever necessary, complete reduction of cytochrome *c* was proven by showing that addition of dithionite did not further increase extinction at 550 nm.

For electron yield experiments, thiosulfate was eliminated from Sox enzyme storage buffers by VivaSpin500 ultrafiltration devices (Sartorius, Göttingen) and the amount of thiosulfate was lowered to 0.2–1 nmol per test. In these experiments the concentration of horse heart cytochrome *c* was 20 μM based on an empirically determined ε of 14.0 cm^2 μmol^{-1}.

2.3. Rhodanese, thiosulfate reductase and 3-mercaptopyruvate:cyanide sulfur transferase assays

To determine whether the rhodanese-like SoxL protein exhibits thiosulfate:cyanide sulfur transferase (rhodanese) activity, assays were conducted according to Ray et al. [14], Papenbrock and Schmidt [15] and Adams et al. [16]. 3-Mercaptopyruvate:cyanide sulfur transferase tests and thiosulfate reductase tests were conducted according to Papenbrock and Schmidt [15].

2.4. Quantification of thiosulfate by HPLC

Fifty microliters aliquots of enzyme reactions (total volume 3 mL) were taken every 10 min for 30 min and processed using the methods of Rethmeier et al. [17] to enable subsequent thiol analysis by HPLC. The concentration of *A. vinosum* SoxYZ in these reaction mixtures was 1 μM.

3. Results and discussion

3.1. Basic enzyme kinetics: SoxYZ

In a first approach, hybrid enzyme assays with *A. vinosum* SoxYZ and *P. pantotrophus* SoxB, SoxXA and Sox(CD)$_2$ were performed. To achieve a noticeable reduction rate of horse heart cytochrome *c*,

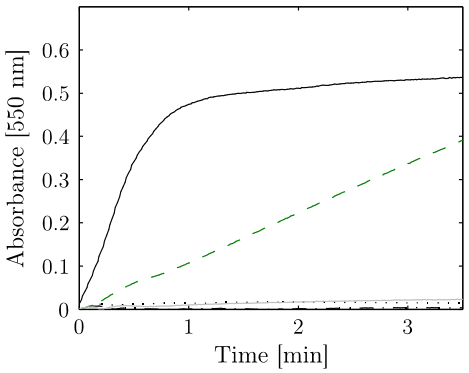

Fig. 2. Enzyme activity of the hybrid Sox enzyme system. *Paracoccus* SoxXA, SoxB and Sox(CD)$_2$ (0.5 μM each) were combined with *A. vinosum* SoxYZ (2.5 μM). Solid line: + Sox(CD)$_2$; dashed line: – Sox(CD)$_2$; dotted line: – SoxXA; grey line: – SoxB; and dash-dotted line: – *A. vinosum* SoxYZ. Reactions were run at pH 6.0.

presence of the enzymes SoxYZ, SoxB and SoxXA was absolutely essential. Control reactions in which one of these Sox enzymes was omitted showed activities below 0.3 mU, The non-enzymatic reduction rate of cytochrome *c* by thiosulfate was 6.6×10^{-3} mU. Additional presence of Sox(CD)$_2$ from *P. pantotrophus* led to an about 5-fold increase of the cytochrome *c* reduction rate (Fig. 2) which correlated roughly with the expected 4-fold increase in electron yield at a constant turnover of thiosulfate (Fig. 1A).

Table 1
Effect of pretreatment of SoxYZ with Na_2S, DTT or $Na_2S_2O_3$ on the specific thiosulfate-oxidizing activity of the Sox enzyme system.[a]

Chemical for pretreatment	Relative activity of the Sox enzyme system	
	P. pantotrophus SoxYZ (0.5 μM)	*A. vinosum* SoxYZ (2.5 μM)
None	1.0	1.0
Na_2S	24.2	2.3
DTT	9.8	0.3
$Na_2S_2O_3$	2.1	0.3

[a] Control reactions were performed with SoxYZ "as isolated". Values for *P. pantotrophus* SoxYZ are taken from [11]. For this organism, the specific activity of the reconstituted Sox enzyme system with SoxYZ "as isolated" was 0.011 U (mg protein)$^{-1}$. When *P. pantotrophus* SoxYZ was replaced with *A. vinosum* SoxYZ "as isolated", the specific activity was 0.038 mU (mg protein)$^{-1}$. The "as isolated" SoxYZ proteins were pretreated with the respective compounds for 20 min at 30 °C and washed three times. These preparations were then used to reconstitute the Sox enzyme system with the other "as isolated" Sox proteins from *P. pantotrophus* to give a final concentration of SoxB, SoxXA and Sox(CD)$_2$ of 0.5 μM each. The degree of activation of recombinant *A. vinosum* SoxYZ was approximately the same for three independent preparations.

As already described for SoxYZ from *P. pantotrophus* [13], full activity of the recombinant *A. vinosum* SoxYZ required activation by treatment with sulfide. The degree of activation by sulfide was 2.3-fold when determined in reaction mixtures containing 0.5 μM SoxXA, SoxB and Sox(CD)$_2$ of *P. pantotrophus*, and 2.5 μM SoxYZ of *A. vinosum* (Table 1). Thus, recombinant "*A. vinosum* SoxYZ as isolated" appeared to be in a relatively more active form as compared to the *P. pantotrophus* enzyme, for which an activation factor of 24.2 has been documented [13]. In contrast to the latter, *A. vinosum* SoxYZ was not activated by treatment with dithiothreitol (DTT) or thiosulfate but was deactivated by about 70% (Table 1).

In reaction mixtures with *P. pantotrophus* SoxXA, SoxB and Sox(CD)$_2$, the cytochrome *c* reduction rate was almost linearly dependent on the concentration of *A. vinosum* SoxYZ (Fig. 3A) reaching a saturation at 3 μM SoxYZ. Hence, *A. vinosum* SoxYZ productively works with *P. pantotrophus* SoxB, SoxXA and even Sox(CD)$_2$ although in the *A. vinosum* Sox enzyme system there is no Sox(CD)$_2$ homolog. This confirms the versatility of SoxYZ and its peptide swinging arm [11] which even allows it to interact with Sox partners from other species.

3.2. Basic enzyme kinetics: SoxB

In a second approach *A. vinosum* SoxB was included in the enzyme assays. *A. vinosum* SoxB did not interact with *P. pantotrophus* SoxYZ but reacted productively with *A. vinosum* SoxYZ (compare dashed and dotted lines in Fig. 4A and B). This was surprising as *P. pantotrophus* SoxB reacted with both the *P. pantotrophus* and the *A. vinosum* SoxYZ (compare solid lines in Fig. 4A and B). Nevertheless, our experiments proved a specific interaction between the two *A. vinosum* proteins. The concentration dependency (Fig. 5) showed a saturation at approximately 0.1 μM SoxB. In contrast to the SoxYZ concentration dependencies, a very steep increase of activity was observed at low SoxB concentrations. This strongly supported the enzyme character of SoxB which was suggested to act as sulfate thiohydrolase [18]. When an optimized concentration of 0.05 μM *A. vinosum* SoxB was used in the test system, the concentration dependency of *A. vinosum* SoxYZ changed (Fig. 3B) and showed a saturation of activity at already 2 μM SoxYZ. The protein SoxYZ is regenerated during the reaction cycle. Therefore its concentration affects the rate (Fig. 4) but not the stoichiometry. This is consistent with the current model where SoxYZ acts as substrate binding molecule [11] (Fig. 1).

3.3. Basic enzyme kinetics: depletion of thiosulfate

In vitro the activity of Sox proteins has so far always been followed exclusively by the reduction of horse heart cytochrome *c*.

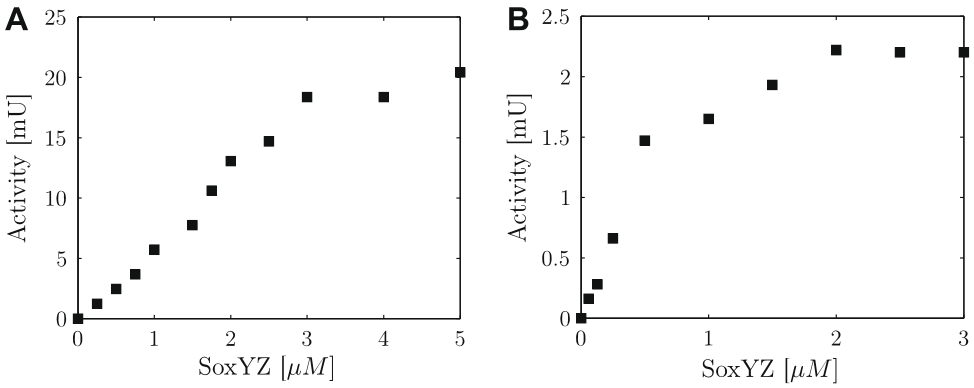

Fig. 3. Dependency on the concentration of *A. vinosum* SoxYZ of the activity of (A) the *P. pantotrophus* enzyme system (SoxXA, SoxB, 0.5 μM each) including 0.5 μM Sox(CD)$_2$ and (B) the hybrid enzyme system (0.5 μM *P. pantotrophus* SoxXA and Sox(CD)$_2$ with 0.05 μM *A. vinosum* SoxB. Reactions were run at pH 7.2.

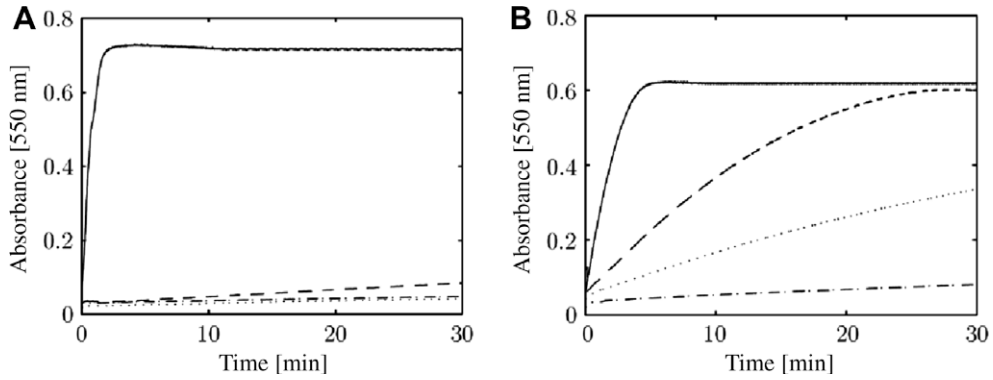

Fig. 4. Activity of the Sox enzyme system combining *A. vinosum* SoxB (0.05 μM), *P. pantotrophus* SoxXA and SoxCD (0.5 μM each) with (A) 0.5 μM *P. pantotrophus* SoxYZ or (B) 2.5 μM *A. vinosum* SoxYZ. Solid line: positive control with addition of 0.5 μM *P. pantotrophus* SoxB; dash-dotted line: negative control without enzymes; dotted line: 0.15 μM *A. vinosum* SoxB; and dashed line: 0.4 μM *A. vinosum* SoxB. Reactions were run at pH 7.2.

Concomitant depletion of thiosulfate has never been experimentally documented. We, therefore, used HPLC analysis for the determination of thiosulfate concentrations in reaction mixtures containing various combinations of Sox enzymes. The data presented in Fig. 6 show that thiosulfate depletion clearly correlated with cytochrome *c* reduction. Under the applied experimental conditions, approximately 5 μM thiosulfate were consumed until the reduction of the cytochrome *c* present in the reaction mixtures reached completion. Without SoxYZ as well as without all Sox enzymes, neither the extinction at 550 nm nor the initial thiosulfate concentration changed.

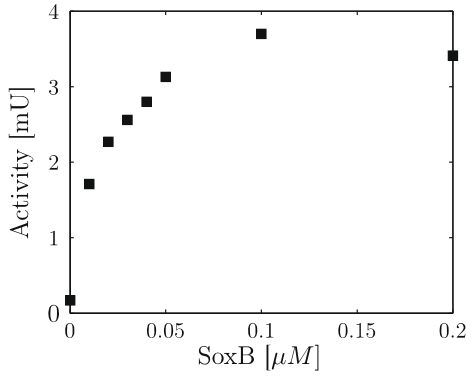

Fig. 5. Dependency of the activity of the complete Sox enzyme system on the concentration of *A. vinosum* SoxB. 2.5 μM *A. vinosum* SoxYZ, 0.5 μM *P. pantotrophus* SoxXA and 0.5 μM Sox(CD)$_2$ were used. Reactions were run at pH 7.2.

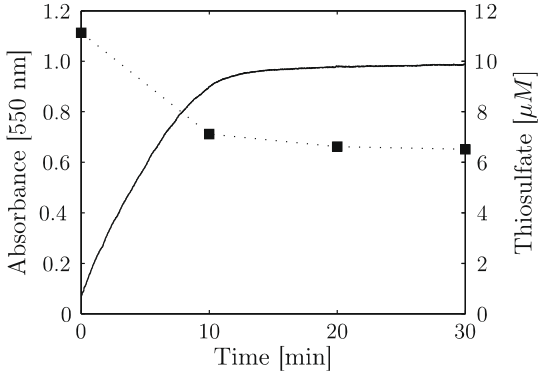

Fig. 6. Correlation between increase of cytochrome *c* reduction (solid line) and thiosulfate depletion (squares) with an initial thiosulfate concentration of 10 μM. A representative experiment is shown. Proteins present: 1 μM *A. vinosum* SoxYZ, 0.5 μM each of *P. pantotrophus* SoxXA, SoxB and Sox(CD)$_2$. Comparative results were obtained with thiosulfate concentrations of 20 μM and 30 μM.

3.4. Electron yields

In electron yield experiments, we elucidated how many moles of electrons were transferred to cytochrome *c* per mol of thiosulfate (Table 2). In order to perform these experiments as reproducible and exact as possible we first empirically determined the molar extinction coefficient (reduced minus oxidized) at 550 nm for the batch of horse heart cytochrome *c* employed in this set of experiments. At 20 μM cytochrome *c* we obtained $\varepsilon = 14.0\ cm^2\ \mu mol^{-1}$. Based on this value, a yield of 9.75 mol electrons per mol thiosulfate was determined in the presence of Sox(CD)$_2$, whereas without Sox(CD)$_2$ a yield of 3.43 mol electrons per mol thiosulfate was calculated. When we based our calculations on the extinction coefficient of 21.1 $cm^2\ \mu mol^{-1}$ given in the literature [12], values of 6.47 and 2.28 were obtained. The latter values match the theoretically expected absolute number of electrons gained in the presence (8 electrons) versus the absence (2 electrons) of Sox(CD)$_2$ better. The observed increase of electron yield upon addition of Sox(CD)$_2$ is consistent with the approximately 5-fold increase of the initial rate of horse heart cytochrome *c* reduction when Sox(CD)$_2$ was added to the reaction (Fig. 2). In these experiments, SoxYZ was present in amounts equivalent to those of the substrate (1.75 nmol versus 0.2–1 nmol thiosulfate).

Table 2
Electron yields in the presence and absence of *Paracoccus pantotrophus* Sox(CD)$_2$.

Sox(CD)$_2$	$S_2O_3^{2-}$ consumed[a] (nmol)	Cytochrome *c* reduced (nmol)[b]	Electron yield (mol cytochrome *c* reduced/mol $S_2O_3^{2-}$)[b]
+	0.2	2.00/1.33	10.0/6.7
+	0.4	3.80/2.52	9.5/6.3
–	0.5	1.75/1.16	3.5/2.3
–	1.0	3.40/2.26	3.4/2.3

Reaction mixtures contained 0.5 μM of *P. pantotrophus* SoxXA and Sox(CD)$_2$ enzymes, 2.5 μM (1.75 nmol in a total volume of 0.7 mL) *A. vinosum* SoxYZ and 0.05 μM *A. vinosum* SoxB.

[a] Difference between thiosulfate added and amount of thiosulfate at the end of the experiment. The final thiosulfate concentration was determined by HPLC analysis and proved to be below the detection limit in all cases.

[b] Amount of cytochrome *c* reduced and electron yields based on an empirically determined $\varepsilon = 14.0\ cm^2\ \mu mol^{-1}$ (first value) or on $\varepsilon = 21.1\ cm^2\ \mu mol^{-1}$ [12] (second value) for horse heart cytochrome *c*.

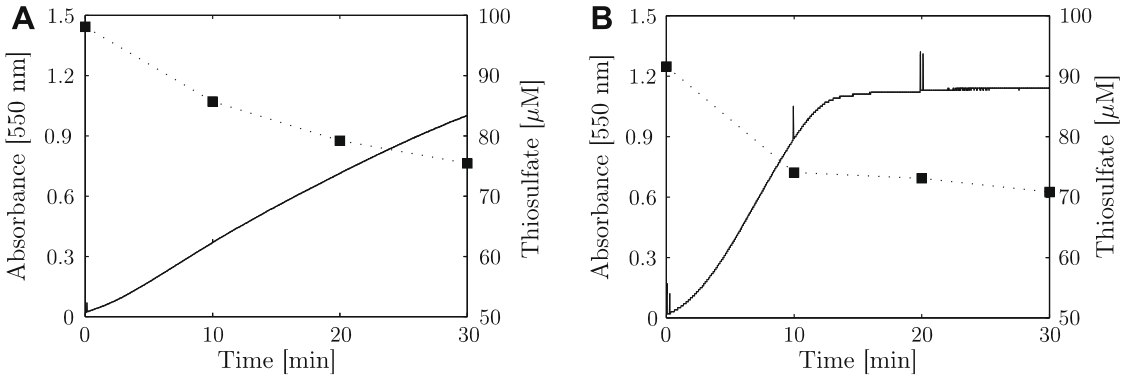

Fig. 7. Correlation of thiosulfate depletion (squares) with cytochrome *c* reduction (solid line) without SoxL (A) and with 1 μM SoxL (B). Other proteins present: 1 μM *A. vinosum* SoxYZ, 0.5 μM *P. pantotrophus* SoxXA and 0.5 μM *P. pantotrophus* SoxB.

3.5. Activity of SoxL

As evident from Fig. 1B, the currently proposed mode of action for the *A. vinosum* Sox system involves the transfer of SoxY-bound sulfane sulfur to growing sulfur globules by one or more sulfurtransferase-catalyzed steps. One likely candidate for catalyzing such a reaction is the rhodanese-like protein encoded immediately downstream of the *soxXAK* genes in *A. vinosum*. Due to the presence of a signal peptide-encoding sequence the protein is predicted to reside in the periplasm, i.e. in the same cellular compartment as the other Sox proteins and also the sulfur globules [2]. When analyzing the active site loop sequences of a huge number of proteins belonging to the rhodanese superfamily, Bordo and Bork [19] recognized several distinct groups. According to their analyses active thiosulfate:cyanide sulfurtransferases are characterized by a CRXGX[R/T] motif. The sequence CNGIWCP in the *A. vinosum* SoxL protein does not exactly match this motif but is rather similar to the so-called HTH-ArsR motif [CRGXYCX]. In line with this reasoning, we have found that the recombinant *A. vinosum* rhodanese-like protein did not show thiosulfate:cyanide sulfur transferase, 3-mercaptopyruvate:cyanide sulfur transferase or thiosulfate reductase activity in vitro independent on whether the protein was directed to the *E. coli* periplasm or cytoplasm. These findings indicate that this protein is not able to act on free thiosulfate as substrate.

However, when we included the protein in Sox enzyme assays containing SoxXA from *P. pantotrophus* and SoxYZ and SoxB from *A. vinosum*, thiosulfate consumption velocity significantly increased in comparison to the control lacking the protein (Fig. 7): In reaction mixtures initially containing 100 μM thiosulfate, 17.5 μM were consumed during the first ten minutes in the presence of SoxL while thiosulfate concentration decreased by only 12.4 μM during the same period of time in its absence. The rate of cytochrome *c* reduction developed reciprocally to thiosulfate consumption and was significantly higher in the presence of SoxL (compare solid lines in Fig. 7A and B). Theoretical considerations would exclude a higher electron yield per thiosulfate but rather predict a faster substrate turnover in the presence of a sulfur transferase with the potential to relieve the active site cysteine of SoxY from an excess of bound sulfur atoms (Fig. 1B). Along this line, the total amount of cytochrome *c* reduced per thiosulfate after cessation of the reaction due to complete reduction of cytochrome *c* should not be altered in the presence of a suitable sulfur transferase. This was indeed the case: in the presence of SoxL, 20.8 μM of thiosulfate were depleted when cytochrome *c* reduction reached completion, while we measured a decrease of 21.6 μM in the absence of SoxL under the same experimental conditions. These findings are in sharp contrast to observations made for reactions with and without Sox(CD)$_2$. Presence of the latter enzyme clearly increases the rate of cytochrome *c* reduction not by a higher substrate consumption rate but by a substantial increase of electrons gained per substrate molecule oxidized [6] (see also Table 2). In summary, we conclude that a second oxidation step is clearly not performed by the rhodanese-like SoxL protein from *A. vinosum*. In its presence, the electron yield per thiosulfate remains constant. Instead, our findings strongly argue for a faster recycling of SoxYZ by transfer of one or more sulfur atoms away from its substrate binding cysteine residue in the presence of the rhodanese-like protein SoxL. As SoxL does not act stoichiometrically (1 μM SoxL versus 20 μM thiosulfate), it is reasonable to assume that sulfur chains may first build up on the protein's active site cysteine and that these may be released at some point during the reaction. We therefore tried to detect polysulfides after derivatization with monobromobimane and HPLC analysis. However, this was not successful. Polysulfides of different chain length are in an equilibrium with each other, with longer chains being more stable at lower pH values. At pH values close to 7.0 as in the reaction mixtures here, polysulfide solutions decompose into H_2S and S_8 [20]. When we assume that about 50% of the 20 μM thiosulfate sulfane sulfur is finally transformed into S_8 by this purely chemical reaction, and the rest is present as polysulfides with an average chain length of 6, we would get about 1.25 μM cyclic elemental sulfur and 1.7 μM polysulfides which is below the detection limit of the HPLC methods applied [17]. It will be an important and challenging task for the future to unambiguously identify the proposed reaction products.

In summary, we identified a new protein that interacts with SoxYZ of *A. vinosum* and to which we can assign a sulfur transferase function not only on the basis of amino acid sequence data but also on enzyme assays in vitro. As the protein is encoded in immediate vicinity of and also in the same orientation as the genes *soxXAK* we propose the name SoxL for this protein.

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