



Article

Initial Step of Selenite Reduction via Thioredoxin for Bacterial Selenoprotein Biosynthesis

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Supplementary Materials

<i>Escherichia coli</i>	1	- - - - M S D K I I H L T D D S F D T D V L K A D G A I L V D F W A E W	C	G	P	C	36
<i>Pseudomonas stutzeri</i> F2a	1	- - - - M S D Y I N N V S D S S F E Q D V L Q A D G P V L V D Y W A E W	C	G	P	C	36
<i>Nitrosomonas europaea</i>	1	- - - - M S Q H I H Y V T D A S F E S E V L Q C P V P V L V D Y W A E W	C	G	P	C	36
<i>Rhodospirillum rubrum</i>	1	- - - - - - - M K Q V S D A S F E E D V L K A D G P V L V D F W A E W	C	G	P	C	32
<i>Thermodesulfobacterium commune</i>	1	- - - - - - - M G A P K V T D E T F E T E V L K S P I P V L V D F W A A W	C	G	P	C	34
<i>Helicobacter pylori</i>	1	- - - - - - - M S H Y I E L T E E N F E S T I - - K K G V A L V D F W A P W	C	G	P	C	33
<i>Thermus thermophilus</i>	1	- - - - - - - M A K P I E V T D Q N F D E T L G Q H P - L V L V D F W A E W	C	A	P	C	34
<i>Prochlorococcus marinus</i>	1	- - - - - - - M S S A A A V T D S S F E Q E V L Q S D L P V L V D F W A P W	C	G	P	C	35
<i>Mycobacterium tuberculosis</i>	1	M T D S E K S A T I K V T D A S F A T D V L S S N K P V L V D F W A T W	C	G	P	C	40
<i>Chlamydia trachomatis</i>	1	- - - - - - - M V Q V V S Q E N F A D S I - - A S G L V L I D F F A E W	C	G	P	C	31
<i>Bacillus subtilis</i>	1	- - - - - - - M A I V K A T D Q S F S A E T - - S E G V V L A D F W A P W	C	G	P	C	32
<i>Escherichia coli</i>	37	K M I A P I L D E I A D E Y Q G K L T V A K L N I D Q N P G T A P K Y G I R G I					76
<i>Pseudomonas stutzeri</i> F2a	37	K M I A P V L D E I A K D Y E G R L K V C K L N I D E N Q E T P P K Y G V R G I					76
<i>Nitrosomonas europaea</i>	37	R M I A P L L D E I A S E Y G D R L K I A K L N I D E N Q S T P Q K Y G I R G I					76
<i>Rhodospirillum rubrum</i>	33	R Q I A P A L E E L A T A L G D K V T V A K I N I D E N P Q T P S K Y G V R G I					72
<i>Thermodesulfobacterium commune</i>	35	R V I A P I D E L A E E F E G K V K V M K L N V D E N P V T P G K Y G I R A I					74
<i>Helicobacter pylori</i>	34	K M L S P V I D E L A S E Y E G K A K I C K V N T D E Q E E L S A K F G I R S I					73
<i>Thermus thermophilus</i>	35	R M I A P I L E E I A K E Y E G K L L V A K L D V D E N P K T A M R Y R V M S I					74
<i>Prochlorococcus marinus</i>	36	R M V S P I V D E I S K D F E G K I K V C K L N T D E N P N V A S Q Y G I R S I					75
<i>Mycobacterium tuberculosis</i>	41	K M V A P V L E E I A T E R A T D L T V A K L D V D T N P E T A R N F Q V V S I					80
<i>Chlamydia trachomatis</i>	32	K M L T P V L E A L A A E L P - H V T I L K V D I D S S P R P A E Q Y S V S S I					70
<i>Bacillus subtilis</i>	33	K M I A P V L E E L D Q E M G D K L K I V K I D V D E N Q E T A G K Y G V M S I					72
<i>Escherichia coli</i>	77	P T L L L F K N G E V A A T K V G A L S K G Q L K E F L D A N L A - - -					109
<i>Pseudomonas stutzeri</i> F2a	77	P T L M L F K N G N V E A T K V G A L S K S Q L A A F L D S N I - - - -					108
<i>Nitrosomonas europaea</i>	77	P T L M I F K N G N I E A T K V G A L S K S Q L T A F V D S H L - - - -					108
<i>Rhodospirillum rubrum</i>	73	P T L M I F K D G Q V A A T K I G A L P K T K L F E W V E A S V - - - -					104
<i>Thermodesulfobacterium commune</i>	75	P T L I I F K N G E P V E V I V G A V S K N T I V N A L N K V L A - - -					107
<i>Helicobacter pylori</i>	74	P T L L F T K D G E V V H Q L V G V Q T K V A L K E Q L N K L L G - - -					106
<i>Thermus thermophilus</i>	75	P T V I L F K D G Q P V E V L V G A Q P K R N Y Q A K I E K H L P A T A					110
<i>Prochlorococcus marinus</i>	76	P T L M I F K G G Q K V D T V V G A V P K A T L S G T I S K H L - - - -					107
<i>Mycobacterium tuberculosis</i>	81	P T L I I F K D G Q P V K R I V G A K G K A A L L R E L S D V V P N L N					116
<i>Chlamydia trachomatis</i>	71	P T L I L F K D G K E V E R S V G L K D K D S L I K L I S K H Q - - - -					102
<i>Bacillus subtilis</i>	73	P T L L V L K D G E V V E T S V G F K P K E A L Q E L V N K H L - - - -					104

Figure S1. Amino acid sequence alignment of thioredoxin homologs from various bacterial phyla. Sequences were aligned using Muscle [1]. The amino acid sequences used in this alignment were obtained from protein databases with locus tags of b3781

(*Escherichia coli*), PszF2a_05700 (*Pseudomonas stutzeri* F2a), NE1034 (*Nitrosomonas europaea*), Rru_A3433 (*Rhodospirillum rubrum*), HL41_06235 (*Thermodesulfobacterium commune*), HP_0824 (*Helicobacter pylori*), TT_C1385 (*Thermus thermophilus*), Pro_1139 (*Prochlorococcus marinus*), Rv3914 (*Mycobacterium tuberculosis*), CT_539 (*Chlamydia trachomatis*), and BSU28500 (*Bacillus subtilis*). Yellow highlight, catalytic Cys residues. Asterisks, conserved residues.

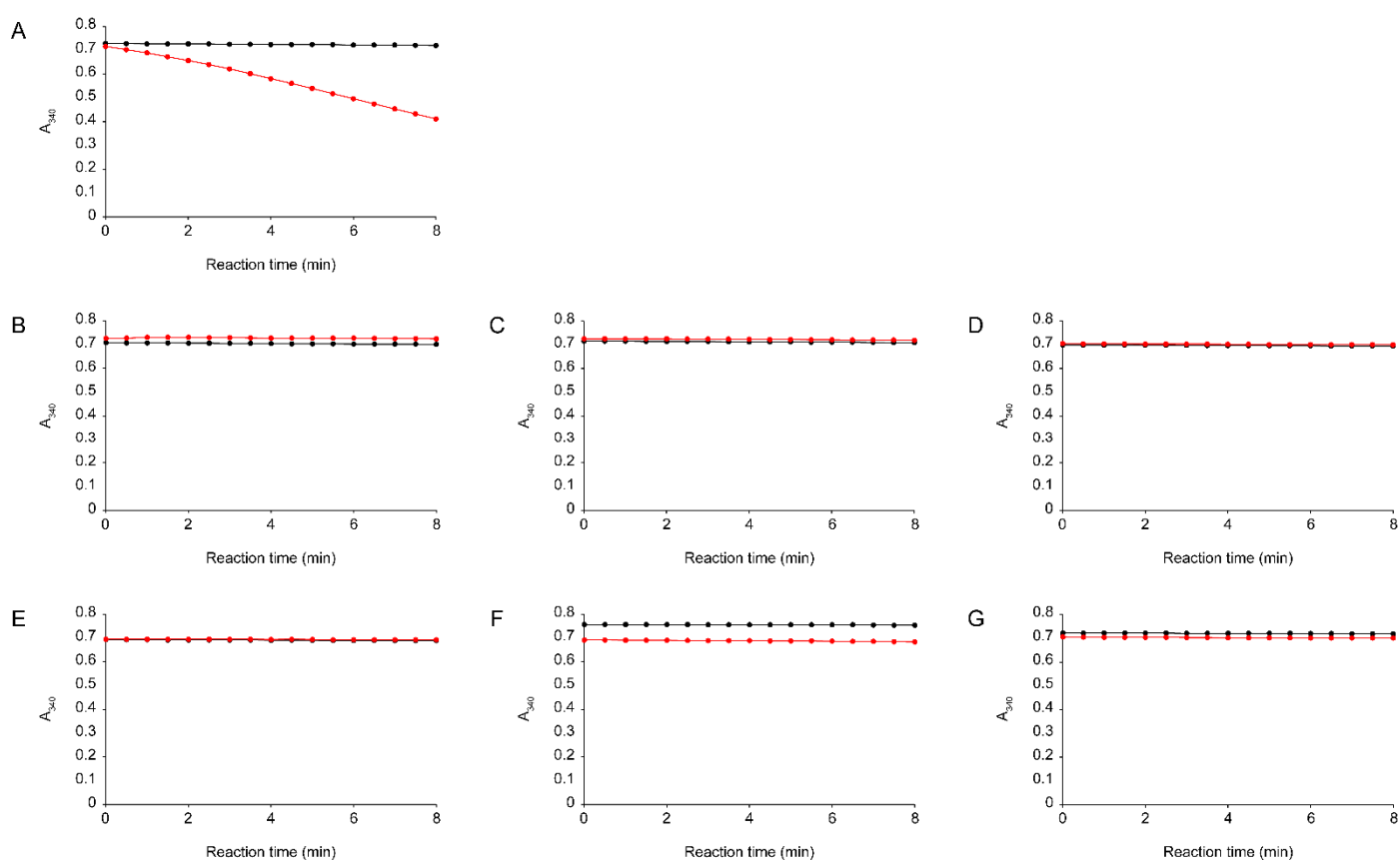


Figure S2. Oxyanion reduction activity of Trx system from *P. stutzeri* F2a. The activity towards selenite (A), selenate (B), sulfite (C), sulfate (D), thiosulfate (E), nitrite (F), and nitrate (G) was measured using PsTrxA with PsTrxR and NADPH by monitoring the decrease in A_{340} due to NADPH oxidation. The assay was performed with (red) or without (black) PsTrxA.

Table S1. Primers used in this study

Primer names	Primer sequences
PsTrxA-f	5'-GGGAATTCCATATGAGCGACTACATCAACAATGTCAGCG-3'
PsTrxA-r	5'-CGCGGATCCTCAGATGTTGCTGTCGAGGAAGGC-3'
PsTrxR-f	5'-GGGAATTCCATATGAGTGAAGTCAAGCATTCGCGTCTG-3'
PsTrxR-r	5'-CGCGGATCCTCAATTGTCCAGGTACTTCTCGGCATC-3'
PsTrxA_C33A-f	5'-ACTGGGCTGAGTGGGCTGGCCCCTGCAAGA-3'
PsTrxA_C33A-r	5'-TCTTGCAGGGGCCAGCCCACTCAGCCCAGT-3'
PsTrxA_C36A-f	5'-AGTGGTGTGGCCCCGCCAAGATGATCGCGC-3'
PsTrxA_C36A-r	5'-GCGCGATCATCTTGGCGGGGCCACACCACT-3'
PsTrxA_C33A/C36A-f	5'-ACTGGGCTGAGTGGGCTGGCCCCGCCAAGA-3'
PsTrxA_C33A/C36A-r	5'-TCTTGGCGGGGCCAGCCCACTCAGCCCAGT-3'

References

1. Edgar, R. C., MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **2004**, *32*, (5), 1792-7.