Hydrogen peroxide inactivates the *Escherichia coli* Isc iron-sulfur assembly system, and OxyR induces the Suf system to compensate.

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Supplementary figures.

S1. The Isc system is the major iron-sulfur cluster assembly system during normal growth. NADH dehydrogenase I is induced when anaerobic cultures are aerated. Activities were measured at intervals after the aeration (at time zero) of wild-type, *ΔhscA*, and *Δsuf* cultures. Note that NADH dehydrogenase I is synthesized in strains lacking the Suf system (*Δsuf*) but not in strains lacking the Isc system (*ΔhscA*)

S2. The *suf* operon is induced by sub-micromolar H_2O_2. Expression after aeration of a transcriptional *lacZ* fusion placed behind the *suf* promoters. Concurrent measurements showed that the H_2O_2 level rose from 0.2 to 0.7 micromolar over the course of this experiment. The specific activity reached only 0.27 in a strain that lacked the OxyR binding site upstream of the *suf* promoter (Fig. 8A).

S3. OxyR is required to induce the Suf system at the sufficient level. Anaerobic Hxp$^-$ Suf$^+$, Hpx$^-$ *suf*^{NI}, and Hpx$^-$ Suf$^-$ cells were diluted into aerobic glucose medium with 6 μM H_2O_2. The *suf*^{NI} allele lacks the OxyR binding site.

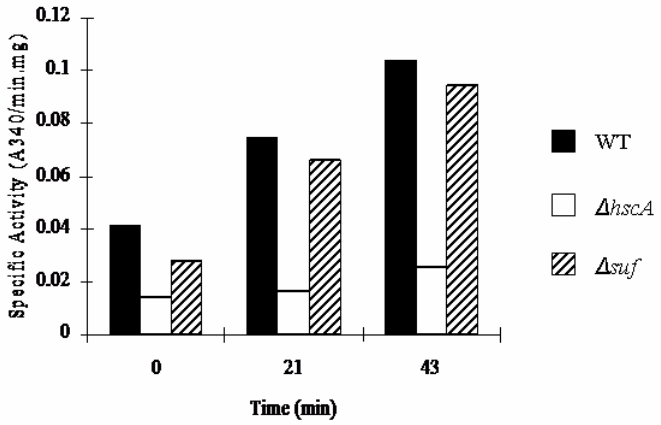
S4. The inactivation of the Isc system is not due to damage to ferredoxin by H_2O_2. (A) Hpx$^-$ cells that overproduce Fdx were harvested anaerobically, concentrated, and then exposed to 0, 10, or 100 μM H_2O_2. Catalase and dithionite were then added to reduce the iron-sulfur cluster to an EPR-visible species. The EPR spectrum of purified Fdx (top) is shown as a control. No significant EPR signal was visible in control strains lacking the *fdx* plasmid (not shown). Note that prior H_2O_2 exposure does not diminish the EPR signal. (B) NADH dehydrogenase I is induced when anaerobic cultures are aerated. Activities were measured at intervals after the aeration of the cultures that were grown in lactose medium. Two independent cultures of Hpx$^-$

Δsuf containing pFdx are presented.

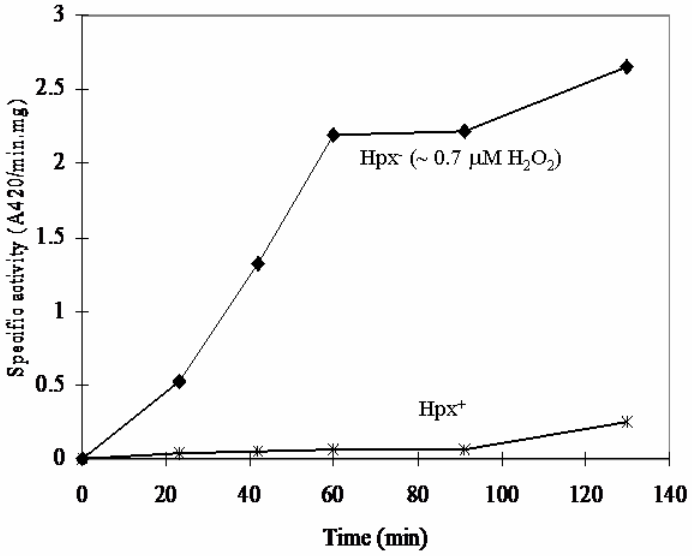
S5. EPR analysis was not able to detect damage to iron-sulfur clusters in the Isc system during H_2O_2 stress. (A) Hpx^- cells that overproduce the Isc system were harvested anaerobically, concentrated, and then exposed to the indicated concentrations of H_2O_2. Note that H_2O_2 does not generated any $[3Fe\text{-}4S]^+$ signal. (B) EPR analysis was also performed after the H_2O_2-exposed cells were treated with catalase and 10 mM dithionite. Prior H_2O_2 treatment did not diminish the EPR signals arising from the reduced Isc protein clusters.

S6. Apo-IscR was accumulated during superoxide stress resulting in the induction of the Suf system. Expression after aeration of transcriptional *lacZ* fusion placed behind either the *suf* promoter (P*suf*) or the suf^{NI} promoter that lacks the OxyR binding site ($Psuf^{NI}$). The Suf induction was mediated by apo-IscR not by OxyR. No significant induction of the Suf system was detected in SOD^+ cells at comparable cell densities (not shown).

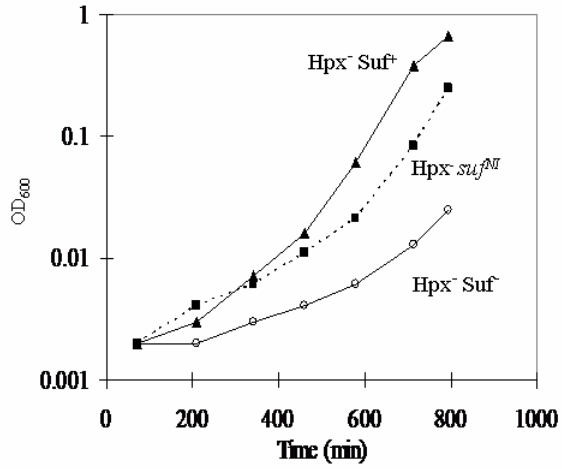
S1


0.12
0.1
0.08
0.06
0.04
0.02
0
Specific Activity (A340/min.mg)
0
21
43
Time (min)
WT
ΔhscA
Δsuf

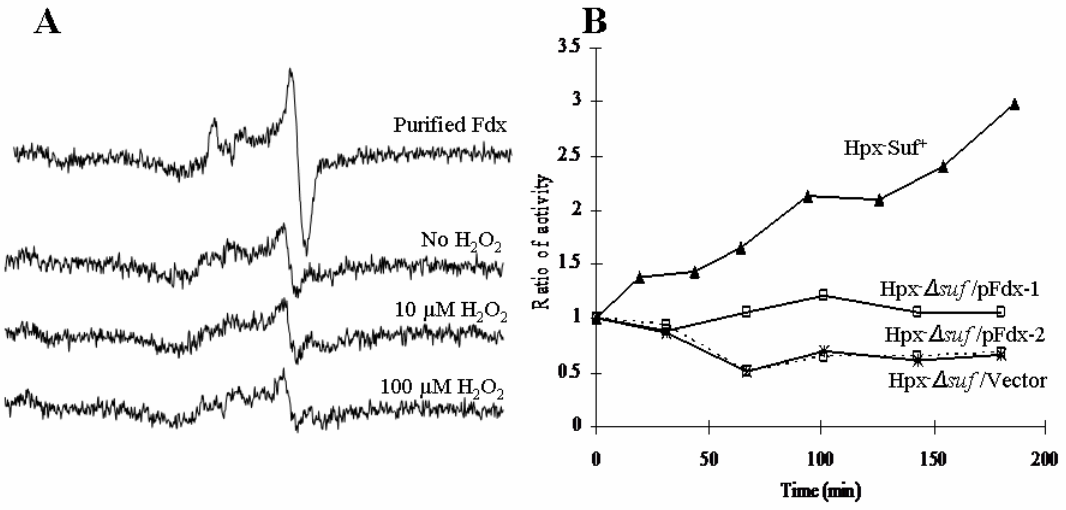
S2


3
2.5
2
1.5
1
0.5
0
Specific activity (A420/min.mg)
0
20
40
60
80
100
120
140
Time (min)
Hpx- (~ 0.7 μM H2O2)
Hpx+

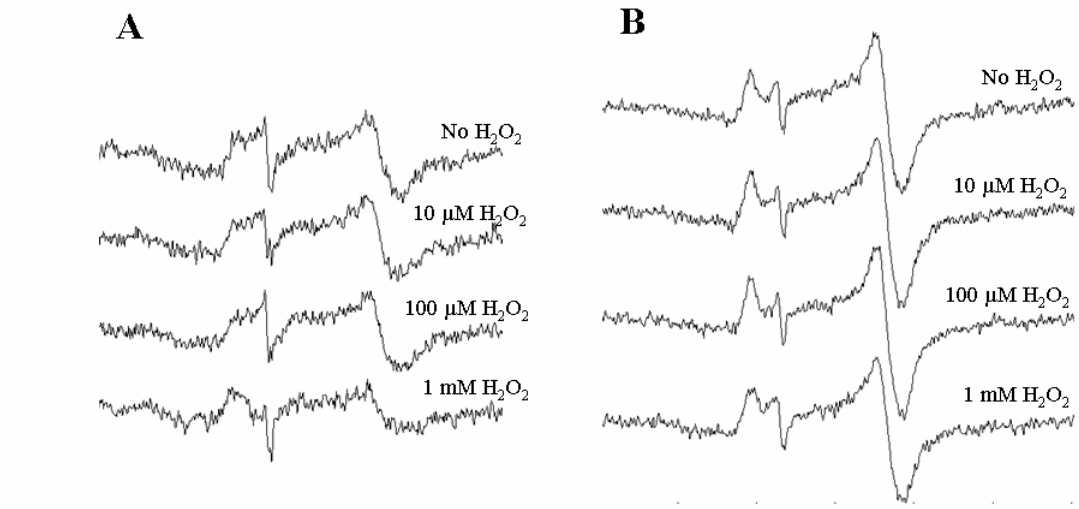
S3


1
0.1
0.01
0.001
OD600
Hpx- Suf+
Hpx- sufNI
Hpx- Suf-
0
200
400
600
800
1000
Time (min)

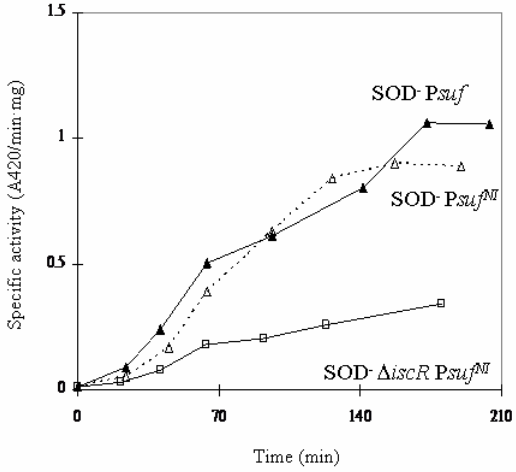
S4


A
Purified Fdx
No H2O2
10 μM H2O2
100 μM H2O2
B
Ratio of activity
Hpx-Suf+
Hpx-Δsuf/pFdx-1
Hpx-Δsuf/pFdx-2
Hpx-Δsuf/Vector
Time (min)

S5


A
No H_2O_2
10 µM H_2O_2
100 µM H_2O_2
1 mM H_2O_2
B
No H_2O_2
10 µM H_2O_2
100 µM H_2O_2
1 mM H_2O_2

S6


1.5
1
0.5
0
0
70
140
210
Specific activity (A420/min·mg)
Time (min)
SOD⁻ P*suf*
SOD⁻ P*suf*^{NI}
SOD⁻ Δ*iscR* P*suf*^{NI}