Supporting Information

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SI Materials and Methods

Growth of *Vibrio cholera* Strains and Bile Salt Survival Assays. All strains were grown at 37 °C to an OD_{600} of ∼0.5, serially diluted, and spot-titered onto LB plates containing various concentrations of either cholate (CHO) or deoxycholate (DOC) or onto MacConkey agar plates. Plates were incubated at 37 °C for 15 h or at 43 °C for 24 h, and colony-forming units were determined.

Determination of Protein Aggregation in Cell Lysates. Cell lysates were prepared by harvesting 200 mL of an overnight culture of MC4100 *ΔrpoH* in LB medium. The cell pellet was dissolved in 10 mL of ice-cold buffer [40 mM KH_2PO_4, 25 mM KCl (pH 7.5)], and cells were lysed by French press. Cell debris was removed by centrifugation (16,000 × *g*, 4 °C, 30 min). The lysate was then subsequently treated with either buffer, 14 mM CHO, or 5 mM DOC at 30 °C for 1 h. The lysates were either directly used or diluted 1:20 into buffer containing no bile salts and incubated for an additional 20 min at 30 °C. Insoluble proteins were separated by centrifugation (16,000 × *g*, 4 °C, 30 min), separated on SDS/PAGE, and visualized using Coomassie stain.

Citrate Synthase Aggregation Measurements. These light-scattering experiments were conducted as described for luciferase and malate dehydrogenase (MDH), except that 12 μM citrate synthase was used.

In Vitro Thiol Trapping of Hsp33. To analyze the thiol status of Hsp33 upon bile salt treatment in vitro, purified Hsp33 was incubated with 14 mM CHO or 5 mM DOC in the presence or absence of 2 mM peroxide at 30 °C for 2 h. Then, an equal volume of 20% trichloroacetic acid (TCA) was added to slow down thiol–disulfide exchange reactions and to precipitate the protein. The protein pellet was resuspended in 20 μL of DAB buffer [200 mM Tris, 6 M urea, 50 mM EDTA, 0.5% SDS (pH 7.5)] supplemented with 15 mM 4-acetamido-4′-maleimidyl-stilbene-2′-disulfonate (AMS) and incubated for 1 h at 25 °C in the dark. Nonreducing Laemmli buffer was added, and the protein was separated on a SDS/PAGE and visualized using Coomassie stain.

Monitoring Protein Carbonylation in Vivo. Wild-type *Escherichia coli* MC4100 was cultivated and treated with bile salts as described above. MC4100 cultivated at 30 °C to mid-log phase and shifted to 43 °C for 30 min (i.e., heat shock) was used as negative control, whereas MC4100 treated with 4 mM hypochlorous acid (HOCl) for 20 min at 37 °C was used as positive control. Protein carbonylation was analyzed according to ref. 1. The protein concentration was determined using the detergent-compatible (DC) protein assay (Bio-Rad). A normalized amount of proteins was loaded onto a 12% reducing SDS/PAGE gel, separated, and transferred to a PVDF membrane for immunoblot detection using specific antibodies against protein hydrazones (DNP) (Sigma-Aldrich).

1. Yan L-J (2009) Protein carbonylation detection. *Curr Protoc Protein Sci* 56:14.4.1–14.4.28.

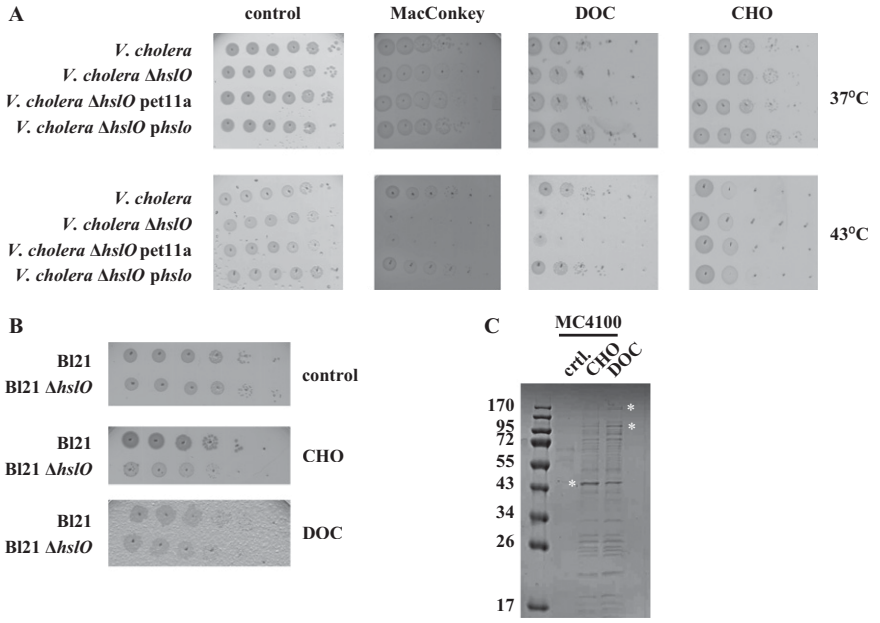

Fig. S1. Hsp33 deletion strains are bile salt-sensitive. (*A*) *Vibrio cholerae* O395, *V. cholerae ΔhslO*, *V. cholerae ΔhslO* pET11a, or *V. cholerae ΔhslO* pET11a-*hslO* were grown at 37 °C to an OD_{600} of 0.5, serially diluted, and spot-titered either onto LB plates containing 7 mM CHO or 3.75 mM DOC or onto MacConkey agar plates. Plates were subsequently incubated at 37 °C for 15 h or at 43 °C for 24 h. (*B*) *E. coli* BL21 and BL21 Δ*hslO* were grown at 37 °C to an OD_{600} of 0.5, serially diluted, and spot-titered onto LB plates containing 20 mM CHO or 23 mM DOC. Plates were incubated at 37 °C for 15 h. (*C*) Lanes 1–4 of SDS/PAGE shown in Fig. 1*D* except that higher-intensity settings were used to make differences in the protein-distribution pattern of cells treated with CHO versus DOC (indicated with a star) more noticeable.

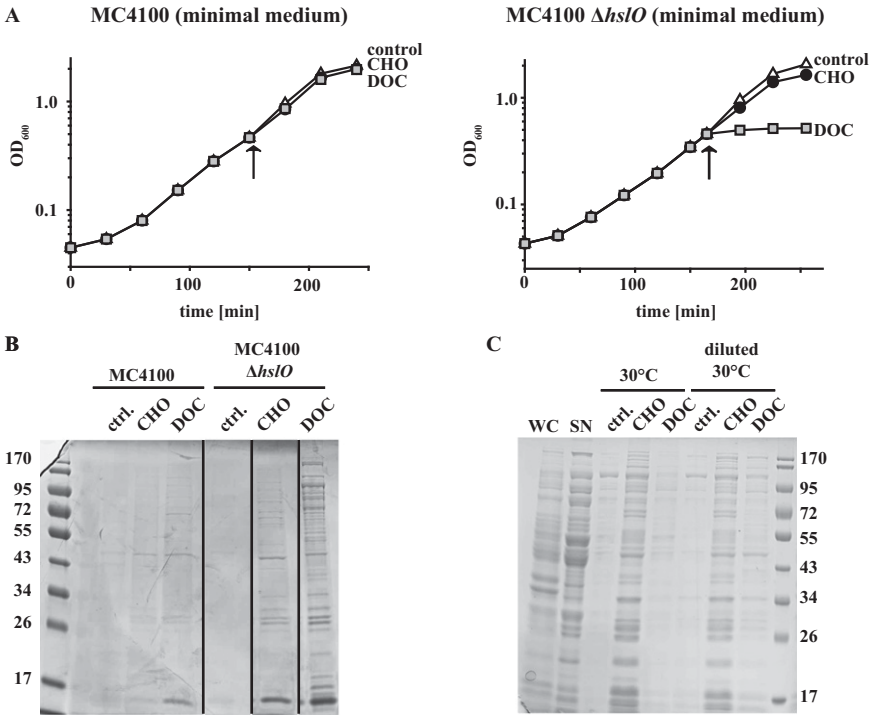

Fig. S2. Bile salts cause protein aggregation in vivo. (*A*) MC4100 or MC4100 *ΔhslO* were grown in 3-(*N*-morpholino)propanesulfonic acid (Mops) minimal media supplemented with amino acids at 37 °C to an OD_{600} of ∼0.5. The arrows indicate the start of treatment with either buffer (open triangle), 42 mM CHO (closed circle), or 7.5 mM DOC (square). Growth was followed for 1.5 h after treatment was started. (*B*) *E. coli* MC4100 and the deletion mutant MC4100 *ΔhslO* were grown in Mops minimal media to an OD_{600} of ∼0.5 and treated with 42 mM CHO or 7.5 mM DOC; 30 min after treatment was started, samples were taken and aggregated proteins were purified for analysis on SDS/PAGE. The amounts of proteins loaded were normalized to OD_{600}. Bars in the gel indicate that some lanes of the gel were deleted for clarity. (*C*) Bile salt treatment leads to aggregation of proteins in cell lysates. A cell lysate of MC4100 was treated with either buffer, 14 mM CHO, or 5 mM DOC at 30 °C for 1 h and either directly spun down and analyzed on 1D gels or diluted 1:20 into buffer containing no bile salt and incubated for additional 20 min at 30 °C. Insoluble proteins were separated by centrifugation, separated on SDS/PAGE, and visualized by Coomassie stain. For comparison, a total cell lysate (WC) and the soluble fraction (SN) of untreated cell lysate was applied on the gel.

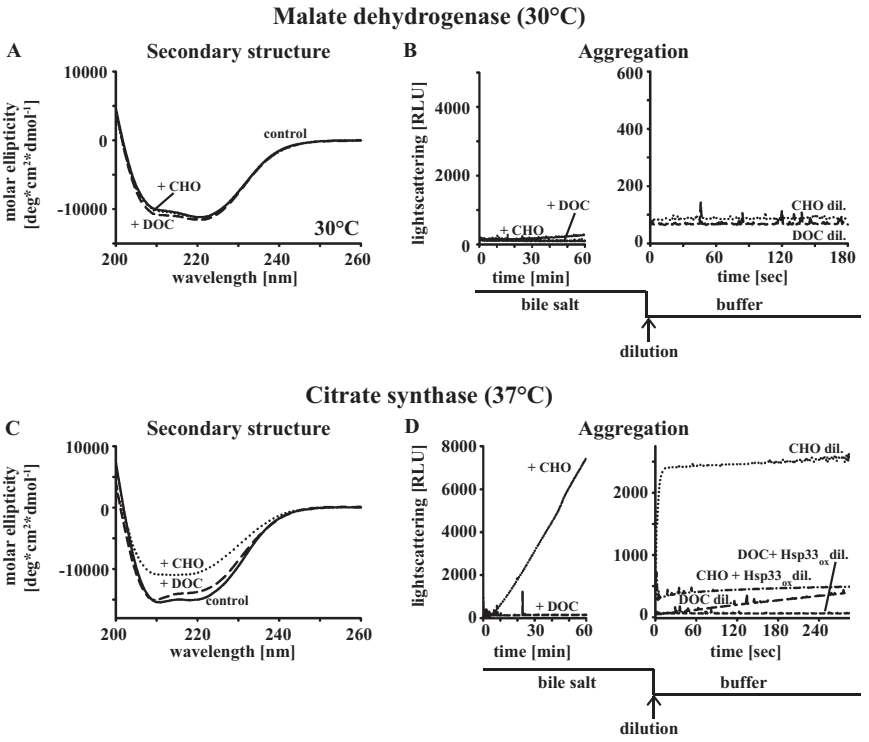

Fig. S3. CHO and DOC are protein-unfolding agents in vitro. (*A*) Influence of bile salts on the secondary structure of MDH at 30 °C. MDH (0.2 mg/mL) was incubated in the absence of bile salts (solid line) or in the presence of either 14 mM CHO (dotted line) or 5 mM DOC (long-dashed line) for 1 h. Then, far-UV–CD spectra were recorded in the presence of the bile salt at the corresponding temperature. All spectra are buffer-corrected. (*B*) Influence of bile salts on the aggregation propensity of MDH at 30 °C. MDH (12 μM) or luciferase was incubated with 14 mM CHO or 5 mM DOC for 1 h at 30 °C. Light scattering was monitored during the incubation or upon a 1:24 dilution of MDH into bile salt-free buffer. (*C*) Influence of bile salts on the secondary structure of citrate synthase at 37 °C. Citrate synthase (0.2 mg/mL) was incubated in the absence of bile salts (solid line) or in the presence of either 14 mM CHO (dotted line) or 5 mM DOC (long-dashed line) for 1 h. Then, far-UV–CD spectra were recorded in the presence of the bile salt at the corresponding temperature. All spectra are buffer-corrected. (*D*) Influence of bile salts on the aggregation propensity of citrate synthase at 37 °C. Citrate synthase (12 μM) was incubated in the presence of 14 mM CHO or 5 mM DOC for 1 h. Aggregation was determined both during the incubation in bile salts and upon dilution of citrate synthase into bile salt-free buffer. To test the effects of Hsp33 on citrate synthase aggregation, a 4-molar excess of activated $Hsp33_{ox}$ was added during the incubation of citrate synthase in CHO or DOC.

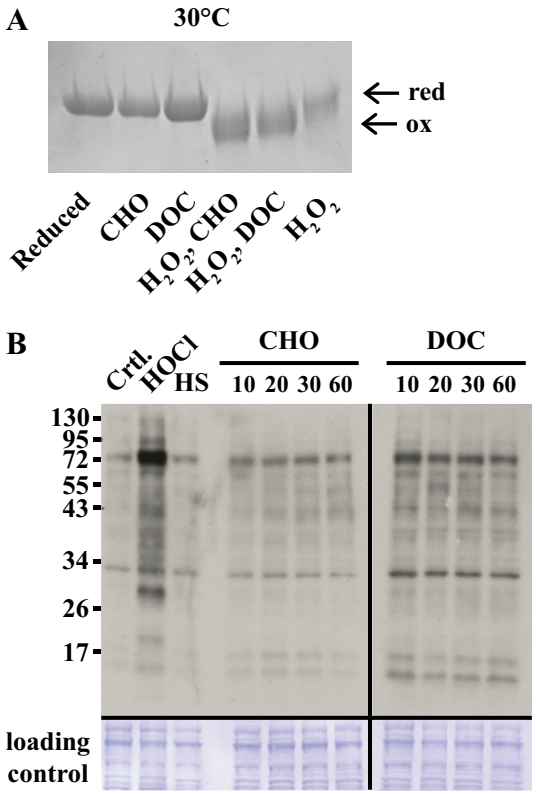

Fig. S4. Bile salts are redox-inactive reagents in vitro but cause oxidative protein carbonylation in vivo. (*A*) In vitro thiol trapping of Hsp33red incubated with 14 mM CHO or 5 mM DOC at 30 °C in the absence and presence of 2 mM H_2O_2. The 490-Da alkylating reagent AMS reacts with all of the reduced cysteines in Hsp33. This alkylation leads to an increase in Hsp33's molecular weight, consistent with its slower migration behavior on SDS/PAGE. (*B*) Cultures of MC4100 were grown in LB to an OD_{600} of 0.5 and split. One aliquot was shifted to 43 °C for 20 min (heat-shock control). The other aliquots were either incubated with 3.5 mM HOCl for 20 min or with 14 mM CHO or 5 mM DOC for the indicated time points at 37 °C. Then, cells were harvested and the carbonylated proteins were derivatized as described. Carbonylated proteins were visualized by immunoblot using antibodies against DPH. The bar indicates that data from two immunoblots, which were processed in parallel, were used for the figure. Bands on the stained PVDF membrane served as loading control.

Table S1. Cellular GSH and GSSG concentrations in bile salt-treated *E. coli*

Experiment	GSH, mM*	GSSG, μM*
Experiment 1		
Control	7.3	110
CHO, 21 mM		
30 min	5.4	135
60 min	7.3	99
DOC, 7.5 mM		
30 min	2.9	143
60 min	6.0	56
Experiment 2		
Control	10.3	126
CHO, 21 mM		
30 min	8.0	126
60 min	9.1	142
DOC, 7.5 mM		
30 min	6.8	90
60 min	6.6	108
Experiment 3		
Control	10.2	145
CHO, 21 mM		
30 min	6.7	133
60 min	6.5	149
DOC, 7.5 mM		
30 min	4.5	125
60 min	6.4	101

Cellular concentrations were calculated using a bacterial cell volume of 1×10^{-15}. GSSG, oxidized GSH.

Table S2. Strains used in this study

Strains	Genotype	Source
E. coli strains		
BL21 DE3	F⁻*ompT gal dcm lon hsdS*$_B$(r_B^- m_B^-) λ(DE3 [*lacI lacUV5-7* gene 1 *ind1 sam7 nin5*])	Lab collection
MC4100	(F-*araD*139 Δ(*argF-lac*) U169 *rspL*150 *relA fl bB*5301 *deoC*1 *ptsF*25 *rbsR) araD+*	Lab collection
DHB4	F′ *lac-pro lacI*^{Q}/*Δ(ara-leu)7697 araD139 ΔlacX74 galE galK rpsL phoR Δ(phoA)*PvuII *ΔmalF3 thi*	Lab collection
MG1655	F-λ- *ilvG- rfb*-50 *rph*-1	Ref. 1
JH17	BL21 *hslO*::K_m	Ref. 2
JW176	MC4100 *hslO*::K_m	Ref. 3
BB7224	MC4100 rpoH::K_m	Ref. 4
LL09	DHB4 *oxyR*::K_m	Lab collection
JH121	DHB4 *rpoH*::tet	Lab collection
A307	DHB4 Δ*trxA*	Ref. 5
AD494	DHB4 *trxB*::K_m	Ref. 6
WP840	DHB4 g*or52...mini-Tn10Tc*	Ref. 6
WP838	DHB4 *grxA*::K_m	Ref. 6
WP823	DHB4 *grxC*::Cm	Ref. 6
MJF152	DHB4 Δ*gshA*::K_m	Ref. 7
MJF155	DHB4 ΔgshB::K_m	Ref. 7
JI377	MG1655 Δ*katG17 katE12*::Tn*10* Δ*ahpCF*′kan::′*ahpF*	Ref. 1
V. cholerae strains		
O395	TCP+ CT+	Lab collection
JW371	O395 *hslO*::Cm	Ref. 8
WC022	O395 *hslO*::Cm pET11	Ref. 9
WC025	O395 *hslO*::Cm pET11A*hslO*	Ref. 9

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Other Supporting Information Files

Dataset S1 (XLSX)