Supplementary Material

Preparation of ^{35}S-lipoate - *E. coli* H-protein encoded by plasmid pNMN108 (1) was mutagenized to change cysteine 59 to serine, removing all cysteines from the H-protein sequence. The resulting plasmid (pLZ003) was transformed into the methionine auxotroph *E. coli* strain B834(DE3) (Novagen). Cells were cultured in a growth medium containing only methionine as a sulfur source. The growth medium was composed of sulfur-free minimal medium (0.83 mM $MgCl_2$, 9.5 mM citric acid, 58 mM K_2PO_4H, 29.7 mM NH_4Cl, 16.7 mM NaH_2PO_4) supplemented with 0.4% (w/v) glucose, 30 µM $FeCl_3$, 50 µg/ml kanamycin, 0.5 mM methionine. When cell density reached 1.0, the expression of the C59S mutant EcH-protein was induced with 0.4 mM IPTG. The cultures were transferred to 20°C, followed by addition of 1 mM octanoate one hour after induction. After an additional 30 minutes, 1 mCi of $Na^{35}SO_4$ (American Radiolabeled Chemicals, 100 mCi/ml) was added, and the culture was maintained at 20°C for an additional 8.5 hours. Cells were then harvested through centrifugation and lysed in BugBuster (Novagen) supplemented with 1 mg/ml lysozyme, and 2.5 µg/ml DNAseI. The cell lysate was cleared by centrifugation and applied to a 1 ml HiTrap Metal Chelating HP column (GE Healthcare) equilibrated with Buffer A (20 mM Na/K phosphate pH 7.5). The metal chelating column was washed with Buffer A containing 50 mM imidazole, followed by connection of an inline 1 ml HiTrap Q FastFlow column (GE Healthcare). Bound protein was eluted from the metal chelating column with Buffer A containing 250 mM imidazole, followed by removal of the metal chelate column. The anion exchange column was then washed with Buffer B (20 mM Na/K phosphate pH 5.0), followed by stepwise elution in Buffer B with increasing concentrations of NaCl. Pure recombinant C59S EcH-protein was precipitated in 10% TCA followed by resuspension in 5 M HCl and incubation at 100°C for 6 hours. The hydrolysate was neutralized with NaOH and buffered in 500 mM Na/K phosphate, pH 7.4. Neutralized hydrolysate was tested for the presence of bioactive *R*-lipoate using the obligate lipoate scavenger strain KER176 *E. coli* (*LipA*$^{-}$) (2). Parallel cultures of KER176 were maintained in lipoate-free minimal medium in the presence of different amounts of hydrolysate or different concentrations of lipoate (Sigma). Culture density was monitored periodically by OD_{600} allowing quantification of *R*-lipoate in the hydrolysate through comparison to cultures with known concentrations of lipoate. The lipoate used in this report contained 0.4 ng/µl of *R*-lipoate with a specific activity of 34.2 Ci/mmol.

1. Cicchillo, R. M., Iwig, D. F., Jones, A. D., Nesbitt, N. M., Baleanu-Gogonea, C., Souder, M. G., Tu, L., and Booker, S. J. (2004) *Biochemistry* **43**, 6378-6386
2. Vanden Boom, T. J., Reed, K. E., and Cronan, J. E., Jr. (1991) *J Bacteriol* **173**, 6411-6420