Supplemental Material

Kinetics of enzymatic mercury methylation at nanomolar concentrations catalyzed by HgcAB

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Supplementary experimental procedures

Identification of RACo homologs in the genomes of Hg methylators

The Basic Local Alignment Search Tool (1) was used to search for homologs of the reductive activator of corrinoid protein (RACo) from *Carboxydothermus hydrogenoformans* (locus tag: CHY_1224, UniProt accession number Q3ACS2, annotated as DUF4445 domain protein or [2Fe-2S] ferredoxin) in selected Hg methylators (*Desulfovibrio desulfuricans* ND132, *Methanosphaerula palustris* E1-9c, *Desulfosporosinus youngiae* JW/YJL-B18, *Dethiobacter alkaliphilus* AHT 1, *Desulfotignum phosphitoxidans* FiPS-3 and *Methanoregula boonei* 6A8) from 3 different groups: Proteobacteria, Firmicutes and Archaea (representative of phylogenetic and metabolic diversity of Hg methylators) (E-value $\leq 1 \cdot 10^{-30}$). The sequence alignment (Fig. S4) was generated using MUSCLE (2).

Free energy of the transition state

The first-order catalytic rate constant (k_{cat}) describes the limiting rate of an enzyme-catalyzed reaction at saturation. Since the overall rate of the reaction is determined by the intermediate with the highest free energy (the transition state), k_{cat} is related to the energy of the transition state (3, 4) as follows:

$$k_{cat} = \frac{k_B T}{h} e^{\frac{-\Delta G^{\ddagger}}{RT}}$$

where k_B is the Boltzmann constant, T is the absolute temperature, h is the Planck constant, R is the universal gas constant and $\Delta G^{\ddagger}$ is the relative free energy of the transition state.

Thus, for a given k_{cat}, $\Delta G^{\ddagger}$ can be calculated as follows:

$$\Delta G^{\ddagger} = RT \cdot \ln \frac{k_B T}{k_{cat} h}$$

Estimates for specific activity

In the absence of studies with purified proteins, we estimate the specific activity of HgcAB based on the following assumptions. The residual amount of MeHg formed in cell lysates exposed to oxygen may be attributable to methylcorrinoids associated with HgcA in the cell lysates prior to oxygen exposure and before the addition of the Hg(II) substrate. Any methylcorrinoids associated with HgcA are not redox-sensitive and should be able to transfer the methyl group to Hg(II), even under aerobic conditions. Once the methyl group is transferred in the presence of oxygen, the corrinoid cannot be reduced back to the Co(I) state. Therefore, the residual amount of MeHg formed after exposure to oxygen and addition of Hg(II) may be the result of a single turnover. We did not observe any significant levels of MeHg in the presence or absence of oxygen in cell lysates of the Δ*hgcAB* mutant, suggesting that Hg methylation by residual methylcorrinoid not associated with HgcA can be ruled out. Assuming that all corrinoid cofactors bound to HgcA are present as methylcorrinoids after exposure to oxygen and prior to the addition of Hg(II), the concentration of MeHg formed in the cell lysates (0.2 nM) should be stoichiometrically equivalent to the concentration of HgcA. Thus, we speculate that the fraction of HgcA is equivalent to approximately 0.0004% (0.2 nM or $7 \cdot 10^{-6}$ mg/mL) of the total protein concentration in cell lysates.

Supplementary Tables

Table S1: Homologs of RACo in the genomes of selected Hg methylators.

Hg Methylator	Group	Locus tag (HgcA, HgcB)	RACo locus tag	% Identity to RACo (CHY_1224)
Desulfovibrio desulfuricans ND132	Proteobacteria	DND132_1056, DND132_1057	DND132_3359	23.88
Desulfotignum phosphitoxidans FiPS-3	Proteobacteria	Dpo_8c00130, Dpo_8c00140	Dpo_4c02960	40.64
Desulfosporosinus youngiae DSM 17734	Firmicutes	DesyoDRAFT_4238, DesyoDRAFT_4237	DesyoDRAFT_4022	48.68
Dethiobacter alkaliphilus AHT 1	Firmicutes	DealDRAFT_3158, DealDRAFT_3157	DealDRAFT_1104	54.99
Methanoregula boonei 6A8	Archaea	Mboo_0422, Mboo_0421	Mboo_1193	39.62
Methanosphaerula palustris E1-9c	Archaea	Mpal_1034, Mpal_1035	Mpal_0806	39.09

Supplementary Figures

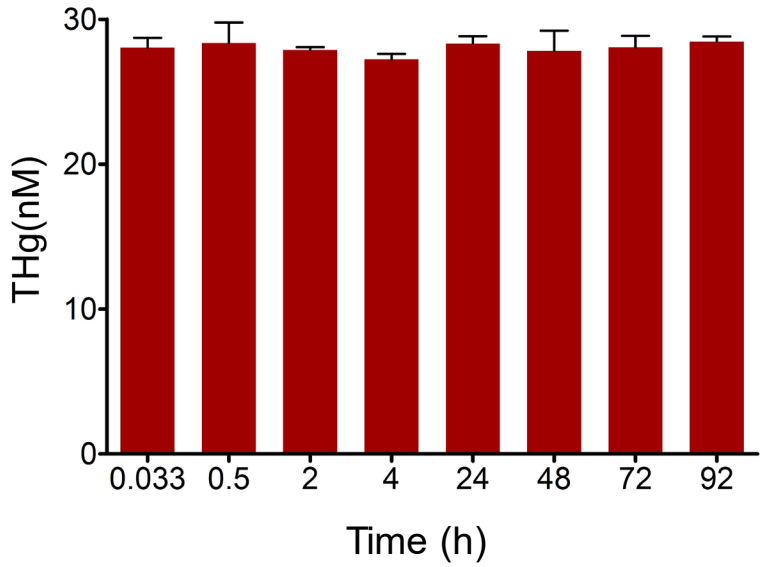

Fig. S1. Total Hg (THg) concentrations for all samples in the time-dependent Hg methylation studies (Fig. 2). (N=2).

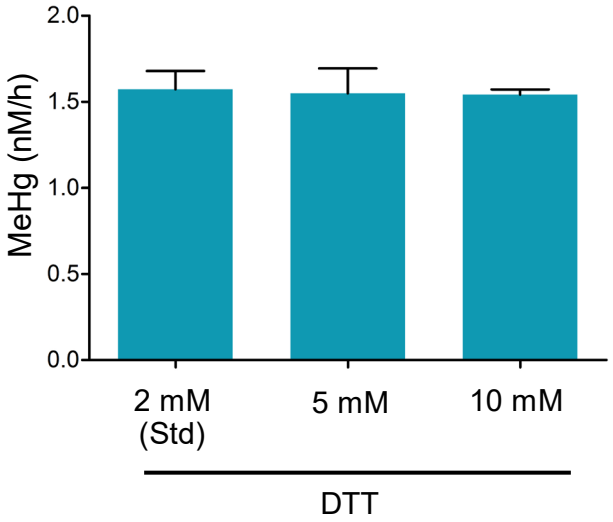

Fig. S2. Effect of DTT concentration on Hg methylation rate in cell lysates of WT ND132.

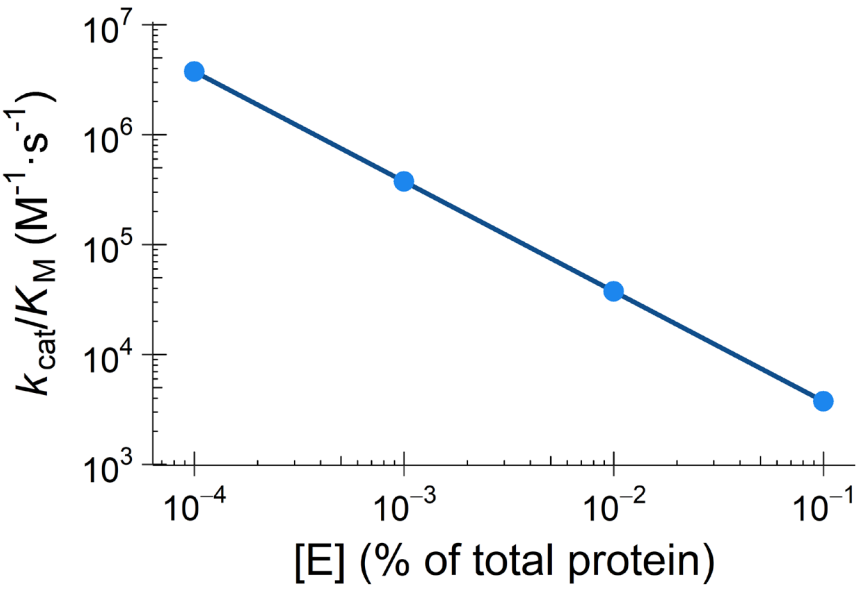

Fig. S3. Catalytic efficiency of HgcAB-mediated Hg methylation. Predicted catalytic efficiencies (k_{cat}/K_M) of HgcAB-mediated enzymatic Hg methylation for a range of postulated enzyme (HgcA) concentrations expressed as a percentage of total cell protein (Table 1).

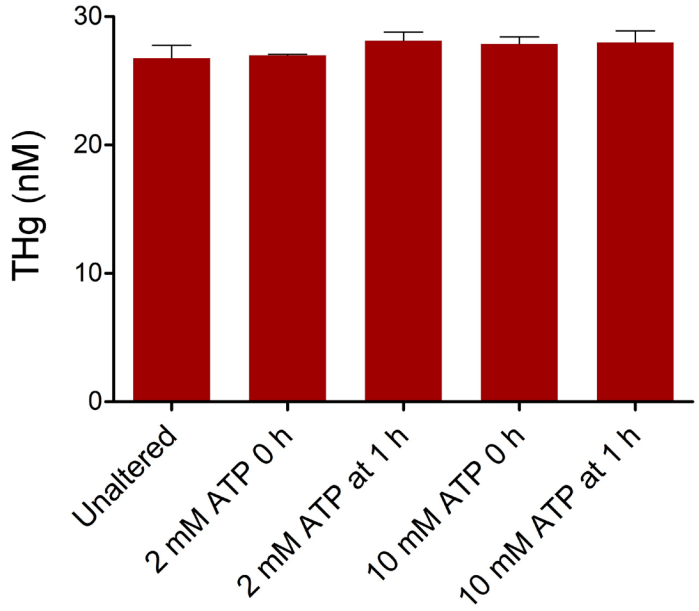

Fig. S4. THg concentrations for all samples in the Hg methylation experiments with addition of ATP (Fig. 5C). (N=2)

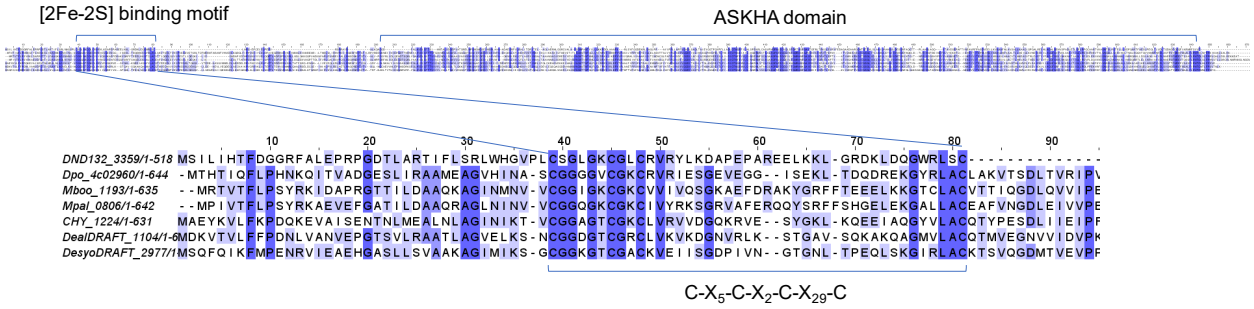

Fig. S5. Multiple sequence alignment (MUSCLE) of the gene encoding the reductive activator of corrinoid protein (RACo) from *Carboxydothermus hydrogenoformans* (CHY_1224) with selected homologs in Hg methylators (Table S1). Conserved residues are indicated in blue, with the shade of blue corresponding to the level of conservation (darker = higher). RACo harbors one [2Fe-2S] binding site and an acetate and sugar kinase/heat shock cognate/actin (ASKHA) domain.

References

1. Altschul SF, Gish W, Miller W, Myers EW, & Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* 215(3):403-410.
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3. Lienhard GE (1973) Enzymatic Catalysis and Transition-State Theory. *Science* 180(4082):149-154.
4. Eyring H (1935) The Activated Complex in Chemical Reactions. *The Journal of Chemical Physics* 3(2):107-115.