Metal active site elasticity linked to activation of homocysteine in methionine synthases

Markos Koutmos^{*†}, Robert Pejchal^{*‡}, Theresa M. Bomer^{*}, Rowena G. Matthews^{§¶}, Janet L. Smith^{§¶}, and Martha L. Ludwig^{*¶**}

^{*}Biophysics Research Division, [§]Life Sciences Institute, and [¶]Department of Biological Chemistry, University of Michigan, Ann Arbor, MI 48109

Edited by Douglas C. Rees, California Institute of Technology, Pasadena, CA, and approved December 31, 2007 (received for review October 18, 2007)

Enzymes possessing catalytic zinc centers perform a variety of fundamental processes in nature, including methyl transfer to thiols. Cobalamin-independent (MetE) and cobalamin-dependent (MetH) methionine synthases are two such enzyme families. Although they perform the same net reaction, transfer of a methyl group from methyltetrahydrofolate to homocysteine (Hcy) to form methionine, they display markedly different catalytic strategies, modular organization, and active site zinc centers. Here we report crystal structures of zinc-replete MetE and MetH, both in the presence and absence of Hcy. Structural investigation of the catalytic zinc sites of these two methyltransferases reveals an unexpected inversion of zinc geometry upon binding of Hcy and displacement of an endogenous ligand in both enzymes. In both cases a significant movement of the zinc relative to the protein scaffold accompanies inversion. These structures provide new information on the activation of thiols by zinc-containing enzymes and have led us to propose a paradigm for the mechanism of action of the catalytic zinc sites in these and related methyltransferases. Specifically, zinc is mobile in the active sites of MetE and MetH, and its dynamic nature helps facilitate the active site conformational changes necessary for thiol activation and methyl transfer.

cobalamin-dependent methionine synthase | cobalamin-independent methionine synthase | methyl transferases | zinc enzymes | zinc inversion

Zinc is a biologically essential metal, vital for the stability of a large number of proteins and for catalysis of many enzymatic reactions (1–5). In enzymes, zinc is found in the +2 oxidation state, Zn(II), which exhibits a d^{10} electron configuration that confers advantageous properties for protein chemistry. These include zinc's lack of redox activity, ability to bind an assortment of ligands because of borderline hardness, and adoption of a variety of coordination numbers ranging from 3 to 8 (6), because a metal with a filled d shell is not subject to geometry-dependent ligand field stabilization energy. Biological zinc sites are usually four-coordinate with distorted tetrahedral geometry, although five- and six-coordinate sites have also been observed. Common protein ligands for zinc centers are His, Glu, Asp, and Cys.

In the majority of catalytic zinc sites, the fourth ligand is a water or hydroxide molecule and gives rise to the common structural and functional motif of $[(X)_3Zn^{2+}(OH)_n]$. For most catalytic zinc sites, the mechanism of action is based on the Zn-(OH)_n functionality; the zinc-bound water is activated for ionization, polarization, or displacement depending on the identity and arrangement of protein ligands. In addition to activation of water, zinc also has been shown to play a role in activating thiols for nucleophilic attack in enzymes such as Ada (7–9), farnesyltransferase (FTase) (10, 11), geranylgeranyltransferase (12), methanol:coenzyme M methyltransferases (13, 14), cobalamin-dependent methionine synthase (MetH) (15, 16), cobalamin-independent methionine synthase (MetE) (17), betaine-homocysteine (Hcy) methyltransferase (18), and nisin cyclase (19, 20). In all of these enzymes, it is thought that direct coordination of the thiol substrate to zinc perturbs its pK_a sufficiently to form an activated thiolate species.

MetE and MetH catalyze the same reaction, the transfer of a methyl group from methyltetrahydrofolate (CH₃-H₄folate) to Hcy to form methionine, but use two very different catalytic strategies. Whereas MetE catalyzes the direct transfer of the N5-methyl group of CH₃-H₄folate to Hcy, MetH utilizes a cobalamin cofactor as an intermediate methyl carrier. The domain organization of both enzymes is uniquely suited to their catalytic strategy: MetE is a simpler enzyme, with binding determinants for both substrates located at the interface of two domains, whereas MetH has a specialized modular architecture consisting of four domains, each with a separate and specific role in catalysis (21). Both enzymes contain a catalytic zinc that coordinates the sulfur of Hcy (15).

Crystal structures of MetEs from *Thermotoga maritima* (22) and *Arabidopsis thaliana* (23) and the N-terminal (1–566) fragment of MetH from *T. maritima* reveal details about the Hcy-binding sites (24). In each enzyme, the zinc site is situated near the top of a $(\beta\alpha)_8$ barrel and is assembled by residues perched at the C termini of inner barrel strands; however, the nature and position of the zinc ligands differs in MetE and MetH, which show no apparent sequence homology. MetE is a member of a larger family of enzymes that display a signature His-X-Cys-X_n-Cys zinc-binding motif, including the coenzyme M (CoM) methyltransferases, methanol:CoM methyltransferase, and epoxyalkane:CoM transferase (25, 26). In contrast, the N-terminal MetH Hcy-binding domain has a zinc center coordinated by three cysteines located in a conserved Asn-Cys-X_n-Gly-Cys-Cys-Gly motif. This domain shares significant sequence and structural homology with betaine-Hcy methyltransferases.

Extended x-ray absorption fine structure (EXAFS) spectroscopy had initially established that the addition of Hcy changes the coordination environment of the zinc in *E. coli* MetH from 3S + 1(N/O) to 4S and from 2S + 2(N/O) to 3S + 1(N/O) in *E. coli* MetE, indicating that binding of Hcy to each enzyme occurs

Author contributions: M.K. and R.P. contributed equally to this work; M.K., R.P., R.G.M., and M.L.L. designed research; M.K., R.P., and T.M.B. performed research; M.K., R.P., J.L.S., and M.L.L. analyzed data; and M.K., R.P., R.G.M., J.L.S., and M.L.L. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Data deposition: The structure coordinates have been deposited in the Protein Data Bank, www.pdb.org [PDB ID codes 3BQ6 for the MetE substrate-free structure; 3BQ5 for the MetE complex with Hcy; 3BOL for the MetH(1–566) substrate-free structure; and 3BOF for the MetH(1–566) complex with Hcy].

[†]To whom correspondence may be addressed at: Biophysics Research Division, University of Michigan, 3050 Chemistry, 930 North University, Ann Arbor, MI 48109-1055. E-mail: mkoutmos@umich.edu.

[‡]Present address: Department of Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037.

[¶]To whom correspondence may be addressed at: 4002 Life Sciences Institute, University of Michigan, 210 Washtenaw Avenue, Ann Arbor, MI 48109-2216. E-mail: rmatthew@umich.edu.

^{**}Deceased November 27, 2006.

This article contains supporting information online at www.pnas.org/cgi/content/full/0709960105/DC1.

© 2008 by The National Academy of Sciences of the USA

by exchange of a resting-state N/O ligand for the sulfur of Hcy, with retention of a tetrahedral zinc environment (27). The crystal structure of the N-terminal fragment of MetH (1–566) from *T. maritima* revealed the expected ligation of the divalent metal ion by Cys-207, Cys-272, and Cys-273 and direct coordination of Hcy sulfur in the binary complex. However, the active site zinc was displaced by cadmium during crystallization, thereby complicating analysis of the metal-site geometry. Importantly, the identity of the exchangeable fourth resting-state zinc ligand (O/N) could not be clearly assigned.

The initial crystal structure of *T. maritima* MetE revealed tetrahedral coordination of zinc in the resting state by His-618, Cys-620, Glu-642, and Cys-704 and implicated Glu-642 as the previously unidentified second low-Z ligand observed by EXAFS. Sequence alignments showed a conserved carboxylate at the Glu-642 position in MetE homologs, suggesting that these enzymes also ligate zinc with four protein ligands in the resting state. However, weak coordination of Hcy, characterized by an unusually long Zn–S(Hcy) distance of ≈ 3.2 Å, was observed in the binary complex with MetE. This complex exhibited distorted trigonal–bipyramidal geometry, with zinc positioned approximately halfway between Glu-642 and Hcy. Because these crystals were obtained under acidic conditions (pH 5.2), we interpreted this structure as a possible transition-state mimic where the Hcy remained protonated, and therefore we anticipated further ligand rearrangement for Hcy to ligate the zinc tightly. We also hypothesized that tight coordination and tetrahedral geometry in the Hcy binary complex could be achieved by further movement of zinc toward substrate, resulting in inversion of geometry about zinc.

In this work, we determined the crystal structures of substrate-free and Hcy-bound *T. maritima* MetE at pH 8.5. Additionally, the crystal structures of a zinc-replete *T. maritima* N-terminal MetH fragment, in the absence and presence of Hcy, are reported. We provide structural evidence for an unusually large zinc displacement in the catalytic cycles of both MetE and MetH, resulting in inversion of metal geometry as Hcy binds and displaces an endogenous ligand from the protein. Our current results provide insight into the role of zinc in MetE, MetH, and homologous zinc-thiol alkyltransferases, bridge the extensive spectroscopic data with the available crystal structures, and provide an unprecedented, to our knowledge, paradigm of metalloenzyme active site behavior.

Results

Structures of MetE. The structure of *T. maritima* MetE with bound Hcy was determined at pH 8.5 to 2.0-Å resolution. Hcy was tightly coordinated to zinc, which displayed tetrahedral geometry with average metal–ligand distances of 2.08 Å to His-618, 2.34 Å to Cys-620 and Cys-704, and 2.31 Å to Hcy (Fig. 1 C and D). These values compared favorably with the distances obtained by EXAFS from the Hcy complex of *E. coli* MetE at pH 7.2 (27). A resting-state structure was also determined at pH 8.5 to 2.1-Å resolution and displayed tetrahedral zinc geometry (Fig. 1 A and B), with bond lengths and angles in agreement with our previously described zinc resting-state structure, as well as with the EXAFS distances determined for substrate-free *E. coli* MetE.

Superposition of the substrate-free and Hcy-bound crystal structures of MetE revealed that the catalytic zinc fully inverted, moving ≈ 1.9 Å (Fig. 2A). The inversion of zinc geometry observed in MetE was enabled by conformational changes in the $\beta 5$ – $\alpha 5$ (L5) loop that carries Tyr-621 and the zinc ligand Cys-620. In the resting form of MetE, the hydroxyl of Tyr-621 formed hydrogen bonds with the backbone amide and carbonyl of Arg-582 and Pro-579, respectively. Pro-579, part of a conserved QIDEPA sequence (22), resides between strand $\beta 4$ and the $\alpha 4A$ extension. Interaction of the invariant Tyr-621 with Pro-579 in the Hcy-free state maintained separation between L5 and $\alpha 4A$.

Fig. 1. Electron density map (A) and ball and stick model (B) showing the metal active site geometry of MetE with no substrates bound (resting state) at 2.1-Å resolution and of MetE with Hcy bound at 2.0-Å resolution (C and D). The blue contours (at 1σ) and red contours (at 6σ) represent electron density from a weighted $2F_{\text{obs}} - F_{\text{calc}}$ map computed after simulated annealing and refinement of the final model. The green contours (at 3σ) represent electron density from a $F_{\text{obs}} - F_{\text{calc}}$ composite-omit map from which the Hcy ligand has been omitted (D). Atom color coding is as follows: gray, carbon; yellow, sulfur; blue, nitrogen; red, oxygen. The unmodeled difference density peak adjoining His-672 is a partially occupied magnesium from the crystallization solution (R.P., unpublished results).

When Hcy was bound and zinc adopted inverted tetrahedral geometry, Tyr-621 rotated into a hydrophobic pocket, where it contacted the side chains of Phe-581, Met-619, Phe-624, Ile-631, and Ile-627. Pro-579 moved into van der Waals contact with the sulfur of Hcy, providing a second hydrophobic interaction in addition to Met-468. The conformational change of L5 is apparently required for full zinc inversion to take place. Therefore the position of Tyr-621 can also be used as an independent check of the location of zinc.

Structures of MetH. Structures of the zinc-replete *T. maritima* N-terminal MetH (1–566) fragment were determined at 1.85-Å resolution, under conditions of physiological (7.0) and acidic pH (4.8, 5.2). The three structures were identical and revealed that zinc in its substrate-free resting state, denoted “Zn_R”, displays approximately tetrahedral geometry. Only the structure at pH 7.0 is reported here. Zinc was coordinated by Cys-207, Cys-272, and Cys-273, with an average Zn–S distance of 2.28 Å (Fig. 3 A and B). This bond length was in close agreement with reported EXAFS values for *E. coli* MetH (27) and expectations for zinc rather than the elongated 2.53-Å average distance observed in the Cd-substituted structures. The coordination of zinc was completed by a fourth ligand, Asn-234, with a Zn–O distance of 2.12 Å. Asn-234 is part of an absolutely conserved signature MetH sequence, PN²³⁴AG. Asn-234 has been identified as a potential fourth ligand in the previously reported N-terminal MetH structure because of its proximity to the metal center (24), although the presence of cadmium instead of zinc in the active site and the long distance between the O of Asn-234 and this Cd center precluded any firm conclusions in the earlier study.

Fig. 2. Structure superposition of the MetE resting state (gray) and the Hcy bound state (green) (A) and of the MetH resting state (gray) and the Hcy bound state (green) (B). Zinc and its ligands are rendered in ball and stick, with colors as described in the legend of Fig. 1. $Zn_{(R)}$, zinc atom at the resting state (no substrates bound); $Zn_{(H)}$, zinc atom at the Hcy-bound state.

In the structure with bound Hcy determined at 1.7-Å resolution at pH 7.0, zinc ($Zn_{(H)}$) was distorted tetrahedral and bound tightly to Hcy, with average metal–ligand distances of 2.32 Å and with distances of 2.35 Å to Cys-207, Cys-272, and Cys-273. The apparent increase in the Zn–S(Cys) distances upon binding of Hcy to MetH was more pronounced than in MetE and was consistent with previously reported EXAFS studies (15). Moreover, the invariant Thr-147 donated a hydrogen bond to the Hcy-δ-S. In the absence of Hcy, Thr-147 was hydrogen-bonded to a water molecule that occupied a position nearly equivalent to that of the Hcy-δ-S.

As in MetE, superposition of the MetH resting-state and Hcy-bound structures revealed that the catalytic zinc moves by ≈ 2.0 Å (Fig. 2B). In MetH, zinc inversion was accompanied by a set of conformational changes centered on the $\beta 8$ – $\alpha 8$ loop that carries Cys-272 and Cys-273. Binding of Hcy recruited helix αB , which appeared to unwind slightly in response to newly formed hydrogen bonds between the carboxyl moiety of Hcy and the backbone amides of residues Tyr-22 and Gly-23. This change brought Thr-24 (the α of Thr-24 was displaced by 1.8 Å) into hydrogen-bonding contact with the backbone amide of Gly-274, favoring a movement of the $\beta 8$ – $\alpha 8$ loop. The loop moved slightly toward Hcy and only partially accounted for the 2.0-Å displacement of zinc, as evidenced by small α – α displacements of Cys-272, Cys-273, and Gly-274, which were 0.4, 0.5, and 0.6 Å, respectively. Dihedral angle adjustments of the Cys-272 and Cys-273 side chains were also necessary to permit the zinc movement.

Discussion

Although MetE and MetH exhibit marked differences in their catalytic mechanisms, they also show striking similarities at the zinc site that appear to be dictated by the chemistry of Hcy activation. A comparison of the MetE and MetH resting states demonstrated that their active site cores, $[Cys_2HisGluZn]$ and $[Cys_3AsnZn]$, respectively, have the same net charge, a fact that had not been appreciated previously. Binding of Hcy was also similar, proceeding through inversion of zinc geometry and displacement of a fourth endogenous ligand: Glu-642 in MetE and Asn-234 in MetH. The identification of Asn-234 as a zinc

ligand in MetH is surprising. Neutral amino acids, such as asparagine and glutamine, are uncommon zinc ligands. They are never found in structural zinc sites, although sometimes they are present in cocatalytic sites (28–30). There is only one known example of a catalytic zinc site with a glutamine ligand: phosphomannose isomerase (31). It is worth noting that water is not a zinc ligand in either MetE or MetH.

An important distinction between the two enzymes is the net charge of the metal site in the binary complex with Hcy, -2 for MetH and -1 for MetE. However, the $Cys_2HisZnHcy$ site of MetE is not necessarily less efficient for activating Hcy than the Cys_3ZnHcy site in MetH, because of the aromatic character of histidine, which has finely tunable electron-donating capabilities. Theoretical calculations predict that zinc-bound histidine has more anionic than neutral character, especially in the presence of negatively charged, electron-donating second shell ligands (32–34). Intriguingly, MetE may have a slightly more negative net charge on the zinc in the transition state if the geometry were more trigonal–bipyramidal than tetrahedral and glutamate were recruited back into the first shell. Relevant structures of the ternary complexes of both enzymes will be needed to fully understand the distribution of charge on the zinc during catalysis, but these will be challenging to obtain because the ternary complexes are not long-lived in either enzyme (35).

Role of Zinc Inversion. Inversion of zinc geometry in both enzymes appears to permit ligation of Hcy by zinc at the face opposite the fourth exchangeable ligand: Glu-642 in MetE and Asn-234 in MetH. One potential role of zinc inversion is to facilitate product release. In both enzymes, the fourth protein ligand could assist product release, coordinating to the zinc and displacing the product after methylation converts the strong Hcy ligand to a weaker methionine ligand.

Moreover, the fourth protein ligand may increase the nucleophilicity of the zinc-thiolate, facilitating methyl transfer, as has been proposed for other zinc enzymes, including FTase (36). The possible mechanism of action for the fourth protein ligand in these enzymes is reminiscent of the valence buffer model proposed for cytidine deaminase (37), shown in Fig. 4A. In cytidine deaminase, the elongation of a Zn–S (Cys) bond results

Fig. 3. Difference electron density (*A*) and ball and stick structural model (*B*) of MetH with no substrates bound (resting state) at 1.85-Å resolution and of MetH with Hcy bound at 1.7-Å resolution (*C* and *D*). The blue contours (at 1σ) and red contours (at 6σ) represent electron density from a weighted $2F_{\text{obs}} - F_{\text{calc}}$ map computed after simulated annealing and refinement of the final model. The green contours (at 3σ) represent electron density from a $F_{\text{obs}} - F_{\text{calc}}$ composite-omit map from which the water in the active site has been omitted (*B*) or from which the Hcy ligand has been omitted (*D*). The atom color coding as in Fig. 1.

in the polarization of a water ligand coordinated to zinc, thus facilitating its deprotonation. The resting-state Zn center in FTase is coordinated in a bidentate fashion to Asp-297b, as well as to His-362b and Cys-299b (11). On binding the substrate peptide, thiol coordination to this center, one of the aspartate oxygens, is displaced. The transition from bidentate to monodentate carboxylate ligand may proceed through a trigonal-bipyramidal state, such as is depicted in Fig. 4B and similar to the one proposed for MetH and MetE depicted in Fig. 4C. In the trigonal-bipyramidal transition states proposed for MetE, MetH, and FTase, the elongation of the Zn–S bond may shift electron density from the zinc to the thiolate sulfur, thus increasing its nucleophilicity and its ability to abstract the methyl group from its corresponding donor. By extension, the role for zinc inversion, discussed in more detail below, may be to permit a similar dynamic interchange between coordinated Hcy and the dissociated thiolate in the enzyme–Hcy binary complex, thus increasing the reactivity of the Hcy thiolate.

Models for Zinc Inversion. How does the zinc in its resting state sense the binding of Hcy from ≈ 4.5 Å away? How does the information propagate to the zinc atom to cause inversion and ligation of Hcy? We can suggest two models to account for inversion: induced fit and dynamic equilibrium.

In an induced fit model, binding of Hcy leads to conformational changes that facilitate zinc displacement. The conformational changes evidenced by our structures are different in MetE

Fig. 4. Role of the dissociable protein ligand to zinc in the catalytic mechanisms of the enzymes cytidine deaminase, farnesyltransferase, and the methionine synthases MetE and MetH. (A) In cytidine deaminase, water is proposed to be activated for attack on C4 of cytidine by lengthening of the bond between Cys-132 and zinc, concomitant with ionization of the water ligand to form hydroxide (37). (B) In farnesyltransferase, the resting enzyme is initially coordinated to His-362b, Cys-299b, and both oxygens of Asp-297b (11). Binding of the peptide substrate is thought to result in formation of a trigonal-bipyramidal transition state because the bidentate coordination of Asp-297 weakens to approach monodentate coordination, referred to as the carboxylate shift mechanism (38, 49). Proton release is associated with peptide binding (10). (C) In MetE and MetH, the nucleophilicity of the coordinated Hcy is proposed to be enhanced by formation of a trigonal-bipyramidal transition state in which the Hcy is partially dissociated and the dissociable protein ligand (Glu-642 in MetE, Asn-234 in MetH) is partially bonded.

and MetH and cannot be directly compared. It is likely that the specific changes induced in each enzyme are a prelude to the formation of the ternary complex, which must have very different and specific structural requirements. Nonetheless, an example of how an induced-fit model may be applied to MetH can be found in the displacement of helix α B, which could act as a spring. Upon binding, the carboxyl moiety of Hcy stretches helix α B and results in the movement of Thr-24, creating a new set of interactions with the β 8– α 8 loop that facilitates displacement of zinc in the direction of substrate (Fig. 5). A direct route of transmission of Hcy-induced conformational changes in MetE is harder to describe. However, the van der Waals contact made by Hcy sulfur with Pro-579 could be sufficiently stabilizing to trigger the conformational changes in L5 and Tyr-621 required for zinc inversion.

In an alternative “dynamic zinc” model, we propose that zinc oscillates between the two tetrahedral geometries of the Zn(R) and Zn(H) states through a trigonal–bipyramidal intermediate even in the absence of Hcy. Three distinct thermodynamic states,

Fig. 5. Structure superposition of the resting state of MetH (gray) and the Hcy-bound state (green). Helix αB moves toward the zinc center upon Hcy binding. Most of the hydrogen bond network remains intact in helix αB , with only minor variations in the distances, but two hydrogen-bonds are lost in the most entatic (stretched) conformation. In an induced-fit model, Thr-24 causes the zinc displacement. The green dashed lines represent hydrogen bonds.

Fig. 6. Equilibrium of the proposed “dynamic” zinc states (A) and energy level graphic depiction of the dynamic zinc model (diagram not drawn to scale) (B).

represented by three different zinc coordination environments, can be populated by zinc to different degrees: (i) tetrahedral with Asn/Glu bound to zinc, Zn(R); (ii) inverted tetrahedral in which zinc is bound to Hcy, or water (the water bound to Thr-147 in MetH) in the absence of substrate, Zn(H); and (iii) an intermediate trigonal-bipyramidal coordination in which the three permanent endogenous ligands form the equatorial plane and the two axial ligands are provided by Hcy/water and Asn/Glu, Zn(I). The d^{10} electron configuration of zinc could allow it to cycle among these geometries without any energetic penalty (no ligand field stabilization energy). These three states, in the absence of substrate, are in equilibrium and populated unevenly (Fig. 6). The addition of Hcy modulates the energy levels of the three states, allowing a redistribution of the conformers that favors zinc inversion. The dynamic nature of the catalytic zinc coupled with substrate-induced conformational changes allows for a much more elastic active site than previously envisioned and is proposed to result in enhanced nucleophilic activation of the zinc-bound Hcy thiolate in the transition state. A similar model for FTase was recently proposed (38).

Metal active sites are usually viewed as relatively static, whereas the conformationally flexible enzyme scaffold is considered dynamic. A new concept has emerged recently in which enzymes are endowed with inherent intrinsic motions, even in the absence of substrates, that are essential for catalysis (39–42). In this paper, we offer a particularly striking example of elasticity

and motion in a metal active site, further extending the concept of the importance of dynamics in catalysis.

Methods

Purification and Crystallizations of MetE. Histidine-tagged *T. maritima* MetE (TM1286-HIS) was cloned and expressed as reported previously (22). Metal affinity-isolated TM1286-HIS was further purified on a monoQ HR16/60 column (GE Healthcare) by elution with a 0–1.5 M KCl gradient.

Crystals were obtained by mixing 2 μ l of 20 mg/ml protein preincubated with 20 mM L-Hcy with 2 μ l of solution containing 19.5% PEG 3350, 200 mM magnesium acetate, 2.5% ethylene glycol, and 100 mM Tris-HCl, pH 8.5. No crystal growth is observed in the absence of added L-Hcy. Crystals were cryoprotected by soaking in a solution of 15% PEG 3350, 125 mM magnesium acetate, 12% ethylene glycol, and 65 mM Tris-HCl, pH 8.5. Substrate-free (resting-state) crystals were obtained by prolonged soaking in cryosolution lacking substrate.

Purification and Crystallizations of MetH. N-terminal (1–566) *T. maritima* MetH was cloned and expressed as reported previously (24). Crystals were obtained by using a modification of the previously reported protocol. To obtain zinc-replete crystals CdCl₂ was replaced with YCl₃. Yttrium does not displace zinc from the active site, but like cadmium, still participates in crystal contacts. Crystals were grown by using the hanging drop vapor diffusion method at 4°C by mixing 2 μ l of 20 mg/ml protein in 50 mM Tris-HCl, pH 8.0, and 1 mM tris(2-carboxyethyl) phosphine with 2 μ l of a solution containing 25% 1,2-propanediol, 10% glycerol, 5% PEG 3000, 100 mM potassium citrate, pH 4.8, 0.5 μ l of 15% 1,2,3-heptanetriol, and 0.5 μ l of 100 mM YCl₃ (final pH, 5.2). L-Hcy cocrystals were obtained by soaking crystals grown in the absence of substrate in a solution containing 20% 1,2-propanediol, 10% glycerol, 5% PEG 3000, 100 mM potassium citrate, pH 5.2, 1.5% 1,2,3-heptanetriol, and 10 mM L-Hcy. Crystals of MetH (with and without Hcy) were obtained by soaking preformed crystals in solutions adjusted to pH 4.8, 5.2, and 7.0.

Structure Determination and Refinement. Diffraction data were collected at the General Medicine and Cancer Institutes Collaborative Access Team (Advanced Photon Source, Argonne National Laboratory) on a Mar300 detector processed with XDS (43) and HKL2000 (44). Phases were obtained by molecular replacement with the program EPMR (45). MetE structures were refined with CNS (46) and PHENIX (47), and both MetH structures were refined with CNS and REFMAC5 (46, 48). In later rounds of refinement, very weak restraints (as minimal as possible) for both the resting and the Hcy-bound state were applied. These restraints were based on ideal tetrahedral geometry with the bond lengths based on appropriate distances for zinc in the +2 oxidation state. Crystallographic information and refinement statistics are provided in [supporting information \(SI\) Table 1](#). Also provided are [SI Movies 1–3](#) illustrating the zinc inversion for both MetH and MetE. The quality of the models was assessed with PROCHECK. PyMOL was used to generate Figs. 1–3 and 5.

ACKNOWLEDGMENTS. We thank Dr. Rebecca Taurog and Kristin Smith for comments on the manuscript and Gail Romanchuk for assistance with protein purification. Diffraction data were collected at the General Medicine and Cancer Institutes Collaborative Access Team at the Advanced Photon Source. This research was supported by National Institutes of Health Grant GM048533 (initially to M.L.L. and currently to J.L.S.).

1. Auld DS (2001) Zinc coordination sphere in biochemical zinc sites. *Biomaterials* 14:271–313.
2. Coleman JE (1992) Zinc proteins: Enzymes, storage proteins, transcription factors, and replication proteins. *Annu Rev Biochem* 61:897–946.
3. Lipscomb WN (1983) Structure and catalysis of enzymes. *Annu Rev Biochem* 52:17–34.
4. Vallee BL, Auld DS (1993) New perspective on zinc biochemistry: Cocatalytic sites in multi-zinc enzymes. *Biochemistry* 32:6493–6500.
5. Vallee BL, Falchuk KH (1993) The biochemical basis of zinc physiology. *Physiol Rev* 73:79–118.
6. Parkin G (2004) Synthetic analogues relevant to the structure and function of zinc enzymes. *Chem Rev* 104:699–767.
7. Myers LC, Cushing TD, Wagner G, Verdine GL (1994) Metal-coordination sphere in the methylated Ada protein-DNA co-complex. *Chem Biol* 1:91–97.
8. Myers LC, Jackow F, Verdine GL (1995) Metal dependence of transcriptional switching in *Escherichia coli* Ada. *J Biol Chem* 270:6664–6670.
9. Myers LC, Terranova MP, Ferentz AE, Wagner G, Verdine GL (1993) Repair of DNA methylphosphotriesters through a metalloactivated cysteine nucleophile. *Science* 261:1164–1167.
10. Hightower KE, Fierke CA (1999) Zinc-catalyzed sulfur alkylation: Insights from protein farnesyltransferase. *Curr Opin Chem Biol* 3:176–181.

11. Park HW, Boduluri SR, Moomaw JF, Casey PJ, Beese LS (1997) Crystal structure of protein farnesyltransferase at 2.25 angstrom resolution. *Science* 275:1800–1804.
12. Zhang H, Seabra MC, Deisenhofer J (2000) Crystal structure of Rab geranylgeranyltransferase at 2.0 Å resolution. *Structure (London)* 8:241–251.
13. Hagemeyer CH, Krer M, Thauer RK, Warkentin E, Ermler U (2006) Insight into the mechanism of biological methanol activation based on the crystal structure of the methanol-cobalamin methyltransferase complex. *Proc Natl Acad Sci USA* 103:18917–18922.
14. LeClerc GM, Grahame DA (1996) Methylcobamide:coenzyme M methyltransferase isozymes from *Methanosarcina barkeri*. Physicochemical characterization, cloning, sequence analysis, and heterologous gene expression. *J Biol Chem* 271:18725–18731.
15. Peariso K, Goulding CW, Huang S, Matthews RG, Penner-Hahn JE (1998) Characterization of the zinc binding site in methionine synthase enzymes of *Escherichia coli*: The role of zinc in the methylation of homocysteine. *J Am Chem Soc* 120:8410–8416.
16. Goulding CW, Matthews RG (1997) Cobalamin-dependent methionine synthase from *Escherichia coli*: Involvement of zinc in homocysteine activation. *Biochemistry* 36:15749–15757.
17. Gonzalez JC, Peariso K, Penner-Hahn JE, Matthews RG (1996) Cobalamin-independent methionine synthase from *Escherichia coli*: a zinc metalloenzyme. *Biochemistry* 35:12228–12234.

18. Millian NS, Garrow TA (1998) Human betaine-homocysteine methyltransferase is a zinc metalloenzyme. *Arch Biochem Biophys* 356:93–98.
19. Li B, et al. (2006) Structure and mechanism of the lantibiotic cyclase involved in nisin biosynthesis. *Science* 311:1464–1467.
20. Okeley NM, Paul M, Stasser JP, Blackburn N, van der Donk WA (2003) SpaC and NisC, the cyclases involved in subtilin and nisin biosynthesis, are zinc proteins. *Biochemistry* 42:13613–13624.
21. Goulding CW, Postigo D, Matthews RG (1997) Cobalamin-dependent methionine synthase is a modular protein with distinct regions for binding homocysteine, methyltetrahydrofolate, cobalamin, and adenosylmethionine. *Biochemistry* 36:8082–8091.
22. Pejchal R, Ludwig ML (2005) Cobalamin-independent methionine synthase (MetE): A face-to-face double barrel that evolved by gene duplication. *PLoS Biol* 3:e31.
23. Ferrer JL, Ravanel S, Robert M, Dumas R (2004) Crystal structures of cobalamin-independent methionine synthase complexed with zinc, homocysteine, and methyltetrahydrofolate. *J Biol Chem* 279:44235–44238.
24. Evans JC, Huddler DP, Hilgers MT, Romanchuk G, Matthews RG, Ludwig ML (2004) Structures of the N-terminal modules imply large domain motions during catalysis by methionine synthase. *Proc Natl Acad Sci USA* 101:3729–3736.
25. Harms U, Thauer RK (1996) The corrinoid-containing 23-kDa subunit MtrA of the energy-conserving N5-methyltetrahydro-methanopterin:coenzyme M methyltransferase complex from *Methanobacterium thermoautotrophicum*. EPR spectroscopic evidence for a histidine residue as a cobalt ligand of the cobamide. *Eur J Biochem* 241:149–154.
26. Krum JG, Ellsworth H, Sargeant RR, Rich G, Ensign SA (2002) Kinetic and microcalorimetric analysis of substrate and cofactor interactions in epoxylance:CoM transferase, a zinc-dependent epoxidase. *Biochemistry* 41:5005–5014.
27. Peariso K, Zhou ZS, Smith AE, Matthews RG, Penner-Hahn JE (2001) Characterization of the zinc sites in cobalamin-independent and cobalamin-dependent methionine synthase using zinc and selenium X-ray absorption spectroscopy. *Biochemistry* 40:987–993.
28. Griffith JP, et al. (1995) X-ray structure of calcineurin inhibited by the immunophilin-immunosuppressant FKBP12-FK506 complex. *Cell* 82:507–522.
29. Kissinger CR, et al. (1995) Crystal structures of human calcineurin and the human FKBP12-FK506-calcineurin complex. *Nature* 378:641–644.
30. Strater N, Klabunde T, Tucker P, Witzel H, Krebs B (1995) Crystal structure of a purple acid phosphatase containing a dinuclear Fe(III)-Zn(II) active site. *Science* 268:1489–1492.
31. Cleasby A, Wonacott A, Skarzynski T, Hubbard RE, Davies GJ, Proudfoot AE, Bernard AR, Payton MA, Wells TN (1996) The x-ray crystal structure of phosphomannose isomerase from *Candida albicans* at 1.7 angstrom resolution. *Nat Struct Biol* 3:470–479.
32. Dudev T, Lim C (2001) Modeling Zn²⁺-cysteinate complexes in proteins. *J Phys Chem B* 105:10709–10714.
33. El Yazal J, Roe NR, Pang YP (2000) Zinc's affect on proton transfer between imidazole and acetate predicted by ab initio calculations. *J Phys Chem B* 104:6662–6667.
34. El Yazal J, Pang YP (2000) Proton dissociation energies of zinc-coordinated hydroxamic acids and their relative affinities for zinc: insight into design of inhibitors of zinc-containing proteinases. *J Phys Chem B* 104:6499–6504.
35. Matthews RG, Smith AE, Zhou ZH, Taurug RE, Bandarian V, Evans JC, Ludwig M (2003) Cobalamin-dependent and cobalamin-independent methionine synthases: Are there two solutions to the same chemical problem? *Helv Chim Acta* 86:3939–3954.
36. Tobin DA, Pickett JS, Hartman HL, Fierke CA, Penner-Hahn JE (2003) Structural characterization of the zinc site in protein farnesyltransferase. *J Am Chem Soc* 125:9962–9969.
37. Xiang S, Short SA, Wolfenden R, Carter CW, Jr (1996) Cytidine deaminase complexed to 3-deazacytidine: A "valence buffer" in zinc enzyme catalysis. *Biochemistry* 35:1335–1341.
38. Sousa SF, Fernandes PA, Ramos MJ (2007) The carboxylate shift in zinc enzymes: a computational study. *J Am Chem Soc* 129:1378–1385.
39. Hammes-Schiffer S, Benkovic SJ (2006) Relating protein motion to catalysis. *Annu Rev Biochem* 75:519–541.
40. Labeikovsky W, Eisenmesser EZ, Bosco DA, Kern D (2007) Structure and dynamics of pin1 during catalysis by NMR. *J Mol Biol* 367:1370–1381.
41. Masgrau L, et al. (2006) Atomic description of an enzyme reaction dominated by proton tunneling. *Science* 312:237–241.
42. Wong KF, Selzer T, Benkovic SJ, Hammes-Schiffer S (2005) Impact of distal mutations on the network of coupled motions correlated to hydride transfer in dihydrofolate reductase. *Proc Natl Acad Sci USA* 102:6807–6812.
43. Kabsch W (1993) Automatic processing of rotation diffraction data from crystals of initially unknown symmetry and cell constants. *J Appl Crystallogr* 26:795–800.
44. Otwinowski Z, Minor W (1997) *Methods Enzymol* 276:307–326.
45. Kissinger CR, Gehlhaar DK, Fogel DB (1999) Rapid automated molecular replacement by evolutionary search. *Acta Crystallogr D* 55:484–491.
46. Brunger AT, et al. (1998) Crystallography & NMR system: A new software suite for macromolecular structure determination. *Acta Crystallogr D* 54:905–921.
47. Adams PD, et al. (2002) PHENIX: building new software for automated crystallographic structure determination. *Acta Crystallogr D Biol Crystallogr* 58:1948–1954.
48. Murshudov GN, Vagin AA, Dodson EJ (1997) Refinement of macromolecular structures by the maximum-likelihood method. *Acta Crystallogr D* 53:240–255.
49. Sousa SF, Fernandes PA, Ramos MJ (2005) Farnesyltransferase—new insights into the zinc-coordination sphere paradigm: Evidence for a carboxylate-shift mechanism. *Biochem J* 388:483–494.