Spectroscopic Study of the Cobalamin-Dependent Methionine Synthase in the Activation Conformation: Effects of the Y1139 Residue and S -Adenosylmethionine on the B 12 Cofactor

ARTICLE *in* JOURNAL OF THE AMERICAN CHEMICAL SOCIETY · NOVEMBER 2008

Impact Factor: 12.11 · DOI: 10.1021/ja8038129 · Source: PubMed

J|A|C|S
ARTICLES

Published on Web 11/12/2008

Spectroscopic Study of the Cobalamin-Dependent Methionine Synthase in the Activation Conformation: Effects of the Y1139 Residue and *S*-Adenosylmethionine on the B_{12} Cofactor

Matthew D. Liptak,[†] Supratim Datta,[‡] Rowena G. Matthews,[‡] and Thomas C. Brunold*,[†]

Department of Chemistry, University of Wisconsin—Madison, Madison, Wisconsin 53706, and Life Sciences Institute and Department of Biological Chemistry, University of Michigan, Ann Arbor, Michigan 48109

Received May 21, 2008; E-mail: brunold@chem.wisc.edu

Abstract: The cobalamin-dependent methionine synthase (MetH) from *Escherichia coli* is a modular enzyme that catalyzes a methyl group transfer from methyltetrahydrofolate to homocysteine via a methylcob(III)alamin (MeCbl) intermediate, generating tetrahydrofolate and methionine (Met). Once every ~2000 turnovers, the cobalamin cofactor is converted to the inactive cob(II)alamin (Co^{2+}Cbl) form, from which MeCbl has to be recovered for MetH to re-enter the catalytic cycle. A particularly puzzling aspect of this reactivation process is that it requires the reduction of the Co^{2+}Cbl species to cob(I)alamin (Co^{1+}Cbl) by flavodoxin, a reaction that would appear to be endergonic on the basis of the corresponding reduction potentials. To explore how MetH may overcome this apparent thermodynamic challenge, we have prepared the I690C/G743C variant of a C-terminal fragment of MetH ($MetH^{CT}$) to lock the enzyme into the activation conformation without perturbing any of the residues in the vicinity of the active site. A detailed spectroscopic characterization of this species and the I690C/G743C/Y1139F $MetH^{CT}$ triple mutant reveals that the strategy employed by MetH to activate Co^{2+}Cbl for $Co^{2+} \rightarrow Co^{1+}$ reduction likely involves (i) an axial ligand switch to generate a five-coordinate species with an axially coordinated water molecule and (ii) a significant lengthening, or perhaps complete rupture, of the $Co-OH_2$ bond of the cofactor, thereby causing a large stabilization of the Co $3d_{z^2}$-based "redox-active" molecular orbital. The lengthening of the $Co-OH_2$ bond is mediated by the Y1139 active-site residue and becomes much more dramatic when the *S*-adenosylmethionine substrate is present in the enzyme active site. This substrate requirement provides MetH a means to suppress deleterious side reactions involving the transiently formed Co^{1+}Cbl "supernucleophile".

1. Introduction

Vitamin B_{12} deficiency, which may be caused by a number of factors, is a serious medical condition whose primary symptoms are megaloblastic anemia and hyperhomocystinuria.[1] The impairment of human methionine synthase (hMS), whose amino acid sequence is 55% identical with that of the *Escherichia coli* cobalamin-dependent methionine synthase (MetH),[2−4] is the primary cause of these symptoms. MetH (like hMS) catalyzes a methyl group transfer from methyltetrahydrofolate (CH_3-H_4folate) to homocysteine (Hcy) via a methylcob(III)alamin (MeCbl) intermediate, generating tetrahydrofolate and methionine (Met).[5] The hMS-catalyzed degradation of CH_3-H_4folate is essential for maintaining the distribution of cellular folate derivatives,[6] as an accumulation of CH_3-H_4folate at the expense of the other cellular folate pools appears to cause megaloblastic anemia (presumably via depletion of the CH_2-H_4folate pool). Improper hMS function also disrupts the biological methylation cycle,[7] leading to Hcy accumulation and hyperhomocystinuria.

Once every ~2000 turnovers, the cobalamin (Cbl) cofactor is converted to the inactive cob(II)alamin (Co^{2+}Cbl) form, from which MeCbl has to be recovered for MetH to re-enter the catalytic cycle.[8] The first, and rate-limiting, step in this activation process is a conformational conversion of MetH into the activation conformation concomitant with cleavage of the axial $Co-N_{H759}$ bond of the cofactor.[9] The structure of the enzyme adopting this conformation was first elucidated by an X-ray crystallographic study of the H759G mutant of a C-terminal fragment of MetH ($MetH^{CT}$; corresponding to residues 649−1227

[†] University of Wisconsin—Madison.
[‡] University of Michigan.

(1) Stabler, S. P.; Allen, R. H. *Annu. Rev. Nutr.* **2004**, *24*, 299–326.
(2) Li, Y. N.; Gulati, S.; Baker, P. J.; Brody, L. C.; Banerjee, R.; Kruger, W. D. *Hum. Mol. Genet.* **1996**, *5*, 1851–1858.
(3) Leclerc, D.; Campeau, E.; Goyette, P.; Adjalla, C. E.; Christensen, B.; Ross, M.; Eydoux, P.; Rosenblatt, D. S.; Rozen, R.; Gravel, R. A. *Hum. Mol. Genet.* **1996**, *5*, 1867–1874.
(4) Chen, L. H.; Liu, M.-L.; Hwang, H.-Y.; Chen, L.-S.; Korenberg, J.; Shane, B. *J. Biol. Chem.* **1997**, *272*, 3628–3634.
(5) Taylor, R. T.; Weissbach, H. *J. Biol. Chem.* **1967**, *242*, 1502–1508.
(6) Banerjee, R. V.; Matthews, R. G. *FASEB J.* **1990**, *4*, 1450–1459.
(7) Matthews, R. G. In *Chemistry and Biochemistry of B12*; Banerjee, R., Ed.; John Wiley & Sons, Inc.: New York, 1999; pp 681−706.
(8) Fujii, K.; Huennekens, F. M. *J. Biol. Chem.* **1974**, *249*, 6745–6753.
(9) Jarrett, J. T.; Hoover, D. M.; Ludwig, M. L.; Matthews, R. G. *Biochemistry* **1998**, *37*, 12649–12658.

10.1021/ja8038129 CCC: $40.75

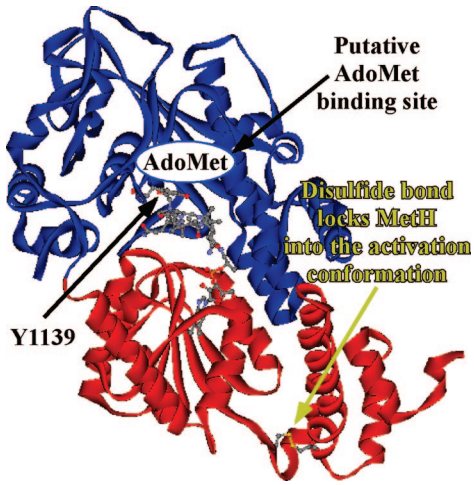


Figure 1. Ribbon representation of I690C/G743C MetH^{CT}, which contains the Cbl-binding (red) and activation (blue) domains, based on the 2.3 Å X-ray crystal structure (PDB file 3BUL).[14] MetH^{CT} is locked into the activation conformation by a disulfide bond between C690 and C743 (gold). The phenolic oxygen of the Y1139 residue and the Co center of the Cbl cofactor are separated by 4.5 Å. The AdoMet-binding site (white) was identified in an X-ray crystallographic study of H759G MetH^{CT} (PDB file 1K98).[10]

of wild-type MetH)[10] that lacks the modules that bind CH_3-H_4folate and Hcy.[11] Following the conformational conversion of MetH into the activation conformation, Co^{2+}Cbl is reduced to cob(I)alamin (Co^{1+}Cbl) by flavodoxin (Fld) in a process that would appear to be endergonic on the basis of the reduction potential of MetH-bound Co^{2+}Cbl and the oxidized/semiquinone and semiquinone/hydroquinone couples of Fld [−490, −260, and −440 mV versus the standard hydrogen electrode (SHE), respectively].[9] Co^{1+}Cbl is then rapidly methylated by *S*-adenosylmethionine (AdoMet) to form the MeCbl product, via a short-lived His-off MeCbl intermediate that lacks the "lower" axial $Co-N_{H759}$ bond.[9]

Studies of wild-type MetH have been complicated by the fact that this enzyme interconverts between at least four nearly isoenergetic conformations,[12,13] which prevents the preparation of homogeneous samples. Thus, to lock the enzyme into the activation conformation without disrupting the interactions made by H759, the I690C/G743C MetH^{CT} variant was constructed.[14] The truncation removes the N-terminal modules that bind and activate CH_3-H_4folate and Hcy,[15] thereby limiting MetH to the resting and activation conformations in which a protective cap and the activation module, respectively, are positioned above the Cbl-binding domain. The disulfide bond introduced between residues 690 and 743 assures that the I690C/G743C MetH^{CT} variant is trapped in the activation conformation, because these residues are located on neighboring secondary structural elements in the activation conformation (Figure 1), but are far apart in the resting conformation. Importantly, the I690C and G743C mutations do not involve any of the residues in the vicinity of the Cbl-binding site.

The motivation for studying a MetH variant locked into the activation conformation that possesses a wild-type-like (i.e., unmodified) Cbl-binding site is 2-fold. First, the interaction of the Y1139 residue and the AdoMet substrate with MetH^{CT}-bound Co^{2+}Cbl can be investigated to evaluate viable strategies employed by the enzyme for catalyzing the thermodynamically challenging reduction of Co^{2+}Cbl to Co^{1+}Cbl. To this end, the Y1139 residue and AdoMet substrate are likely candidates for modulating the properties of the enzyme-bound Co^{2+}Cbl due to their close proximities to the β ("upper") face of the cofactor in the activation conformation (Figure 1),[10,14] in which a water molecule is axially bound to the Co^{2+} ion.[16] Second, this variant allows for the trapping of the initial MeCbl product formed following methyl group transfer from AdoMet to Co^{1+}Cbl. Studies of this species can help determine the relative timing of the final two steps in the activation process, namely, $Co-N_{H759}$ bond formation and conformational conversion of MetH into a catalytic conformation.

In this study, the I690C/G743C and I690C/G743C/Y1139F variants of MetH^{CT} have been characterized by Abs, magnetic circular dichroism (MCD), and electron paramagnetic resonance (EPR) spectroscopies, a set of complementary tools that provide a sensitive probe of the geometric and electronic structures of Co^{2+}Cbl and MeCbl.[17,18] The spectra of MetH^{CT}-bound Co^{2+}Cbl and MeCbl have been analyzed within previously developed computational frameworks to correlate spectral changes with geometric and electronic perturbations of the cofactor.[19,20] This quantitative analysis has afforded a detailed description of the coordination environment of the enzyme-bound Cbl cofactor and revealed that the Y1139 residue and the AdoMet substrate are likely to play a major role in the Co^{2+}Cbl reduction step. Collectively, the results obtained in this study provide significant new insights into the enzymatic strategy employed for Co^{2+}Cbl reduction and the timing of $Co-N_{H759}$ bond formation in the MeCbl-bound product state of the activation process.

2. Materials and Methods

2.1. Chemicals/Cofactors. AdoMet, glycerol, hydrochloric acid (HCl), 2,2,6,6-tetramethylpiperidine-1-oxyl, and MeCbl were purchased from Sigma-Aldrich and used without further purification. Methylcob(III)inamide ($MeCbi^+$) was prepared from MeCbl as described previously.[21]

2.2. Construction of an Expression Plasmid for I690C/G743C MetH^{CT} and I690C/G743C/Y1139F MetH^{CT}. The I690C/G743C double mutation was introduced into the pVB8 vector containing an amino-terminal His_6-tagged *E. coli* wild-type C-terminal MetH, using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). The primers were obtained from Invitrogen Corp. (Carlsbad, CA). The sequence of I690C/G743C

(10) Bandarian, V.; Pattridge, K. A.; Lennon, B. W.; Huddler, D. P.; Matthews, R. G.; Ludwig, M. L. *Nat. Struct. Biol.* **2002**, *9*, 53–56.
(11) Goulding, C. W.; Postigo, D.; Matthews, R. G. *Biochemistry* **1997**, *36*, 8082–8091.
(12) Bandarian, V.; Ludwig, M.; Matthews, R. G. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 8156–8163.
(13) Fleishchhacker, A. S.; Matthews, R. G. *Biochemistry* **2007**, *46*, 12382–12392.
(14) Datta, S.; Koutmos, M.; Pattridge, K. A.; Ludwig, M. L.; Matthews, R. G. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 4115–4120.
(15) Evans, J. C.; Huddler, D. P.; Hilgers, M. T.; Romanchuk, G.; Matthews, R. G.; Ludwig, M. L. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 3729–3736.
(16) Liptak, M. D.; Fleishchhacker, A. S.; Matthews, R. G.; Brunold, T. C. *Biochemistry* **2007**, *46*, 8024–8035.
(17) Stich, T. A.; Brooks, A. J.; Buan, N. R.; Brunold, T. C. *J. Am. Chem. Soc.* **2003**, *125*, 5897–5914.
(18) Stich, T. A.; Buan, N. R.; Brunold, T. C. *J. Am. Chem. Soc.* **2004**, *126*, 9735–9749.
(19) Stich, T. A.; Buan, N. R.; Escalante-Semerena, J. C.; Brunold, T. C. *J. Am. Chem. Soc.* **2005**, *127*, 8710–8719.
(20) Stich, T. A.; Seravalli, J.; Venkateshrao, S.; Spiro, T. G.; Ragsdale, S. W.; Brunold, T. C. *J. Am. Chem. Soc.* **2006**, *128*, 5010–5020.
(21) Dorweiler, J. S.; Matthews, R. G.; Finke, R. G. *Inorg. Chem.* **2002**, *41*, 6217–6224.

MetH^{CT} was confirmed by complete sequencing at the Biomedical Research Core Facility of the University of Michigan. The resulting plasmid was named pSD-3.

The Y1139F mutation was introduced into pSD-3 by ligation of a fragment of DNA from the pCS-15 vector containing the mutation. Briefly, pCS-15 was digested with *Hin*dIII and *Bbv*cI at 37 °C. The 330 bp fragment was purified from an agarose gel and ligated into the similarly digested pSD-3 vector, yielding pSD-4. The presence of this mutation was verified by sequencing at the Biomedical Research Core Facility of the University of Michigan.

2.3. Expression and Purification of I690C/G743C MetH^{CT} and I690C/G743C/Y1139F MetH^{CT}. The enzymes were expressed in cells of *E. coli* Hms174(DE3) (Novagen) and purified by nickel affinity chromatography using a 5 mL Hi-trap and a MonoQ 16/10 column (Amersham Biosciences). Protein concentrations were determined spectrophotometrically after reductive methylation in the presence of AdoMet by using the extinction coefficients of the enzyme-bound MeCbl cofactor (ε_{452}[I690C/G743C]= 10.2 mM^{-1} cm^{-1},[14] ε_{452}[I690C/G743C/Y1139F] = 10.3 mM^{-1} cm^{-1}).

2.4. Reduction, Methylation, and Photolysis of Cbl Bound to MetH^{CT}. I690C/G743C MetH^{CT} and I690C/G743C/Y1139F MetH^{CT} were converted to the MeCbl form through reductive methylation by AdoMet in an electrochemical cell.[22] The Co^{2+}Cbl-bound MetH^{CT} forms were generated via photolysis of the corresponding MeCbl-bound species, as described previously.[22]

2.5. Sample Preparation. Samples of MeCbl and Co^{2+}Cbl bound to I690C/G743C MetH^{CT}, ranging in concentration from 300 to 720 μM, were stored in 10 mM potassium phosphate (KP_i) buffer, pH 7.2, at −80 °C. A 690 μM sample of Co^{2+}Cbl bound to I690C/G743C/Y1139F MetH^{CT} was stored in 10 mM KP_i buffer, pH 7.2, at −80 °C. A 38 mM sample of AdoMet was stored in 1 mM HCl, pH 3.0, at −80 °C.

For low-temperature Abs and all MCD spectroscopic measurements, samples were thawed on ice and mixed with the glassing agent glycerol (50−60%, v/v). The samples were then mixed by vortex, microcentrifuged, incubated at 37 °C for 20 min, loaded into MCD cells (which consisted of two quartz disks separated by a neoprene spacer), and immediately frozen in liquid nitrogen. The MeCbl- and MeCbi$^+$-containing MCD samples were handled under minimal ambient light to prevent photolysis of the Co−C bond. The final concentration of Cbl in each sample ranged from 150 to 276 μM. The final concentration of AdoMet was 844 μM, a 3-fold excess over Co^{2+}Cbl.

For EPR and 310 K Abs spectroscopic measurements, all samples were thawed on ice, mixed by vortex, microcentrifuged, and incubated at 37 °C for 20 min. After collection of the 310 K Abs data, the samples were loaded into quartz tubes and immediately frozen in liquid nitrogen. The final concentration of Co^{2+}Cbl in each sample ranged from 252 to 276 μM. The final concentration of AdoMet was 760 μM, a 3-fold excess over Co^{2+}Cbl.

2.6. Spectroscopy. Abs (310 K) spectra were collected on a Varian Cary 5e spectrophotometer. To accurately determine band shifts between Abs spectra of related species, the corresponding data sets were collected back-to-back using the same quartz cuvette (washed with buffer between measurements) and solvent. Low-temperature Abs and all MCD spectra were collected using a Jasco J-715 spectropolarimeter in conjunction with an Oxford Instruments SM4000-8T magnetocryostat. The MCD spectra presented in this paper were obtained by taking the difference between data obtained at +7 and −7 T to remove the contributions from the natural circular dichroism signal and glass strain. The MCD spectra were deconvoluted into a minimal number of Gaussian bands of fixed width to resolve the individual electronic transitions. The complete parameter sets of the MCD spectral deconvolutions are available in the Supporting Information (Tables S1−S6). The estimated uncertainty in band positions derived from these analyses is 1 nm, as dictated by the accuracy of the Jasco J-715 spectropolarimeter. Over the wavelength range investigated, this value corresponds to 100 cm^{-1} or less. Hence, band shifts of >100 cm^{-1} were considered as being significant.

EPR spectra were collected using a Bruker ESP 300E spectrometer equipped with a Varian EIP model 625A CW frequency counter. The sample temperature was varied using an Oxford Instruments ESR 900 continuous flow liquid helium cryostat regulated by an Oxford ITC4 temperature controller. All spectra were collected with 1 mW of microwave power using a field modulation of 10.5 G and 100 kHz and a time constant of 82 ms. A sample of 10 mM KP_i buffer was used to collect a background spectrum. The SIMPOW6 program, which was developed by Dr. Mark Nilges at the University of Illinois on the basis of the QPOW program,[23] was used to simulate the EPR spectra and extract the spin Hamiltonian parameters. To limit the number of adjustable parameters, the **g** and **A** tensors were assumed to be collinear. The complete parameter sets of the EPR spectral simulations are available in the Supporting Information (Tables S7−S9).

3. Results and Analysis

3.1. The MCD spectrum of MetH^{CT}-bound Co^{2+}Cbl Depends on the Identity of Residue 1139. The catalytically inactive Co^{2+}Cbl form is the initial cofactor state in the MetH activation process in which MeCbl is generated via a Co^{1+}Cbl intermediate by reductive methylation using Fld and AdoMet. Previous spectroscopic studies of H7596 MetH have revealed that, following the rate-limiting conformational conversion of MetH into the activation conformation, the enzyme-bound Co^{2+}Cbl species adopts a five-coordinate, His-off coordination with a water ligand bound axially to the Co^{2+} ion on the β, or upper, face of the cofactor.[16] An excellent model for this five-coordinate, His-off Co^{2+}Cbl species is provided by cob(II)inamide (Co^{2+}Cbi$^+$), a Co^{2+}Cbl derivative that lacks the nucleotide loop and thus binds a water molecule in the axial coordination site originally occupied by the dimethylbenzimidazole (DMB) moiety.[18] Below, the spectroscopic properties of Co^{2+}Cbi$^+$ and MetH^{CT}-bound Co^{2+}Cbl are compared to assess the degree by which the enzyme active site and/or the AdoMet substrate perturb the structure of the five-coordinate, His-off Co^{2+}Cbl intermediate.

The MCD spectra of free Co^{2+}Cbi$^+$ and several MetH^{CT}-bound Co^{2+}Cbl species are compared in Figure 2. Parallel studies were also carried out using Abs spectroscopy (Figure S1, Supporting Information); however, because MCD spectroscopy has proven to offer a much more sensitive probe of small changes in the axial coordination environment of Co^{2+} corrinoid species, only the MCD data provided in Figure 2 are discussed here.[16,18−20,24] Our spectral analysis of Co^{2+}Cbi$^+$ completed previously revealed that the electronic transitions observed by MCD spectroscopy can be classified as Co 3d → 3d transitions, Co 3d → corrin π^* charge transfer (CT) transitions, and corrin $\pi \rightarrow \pi^*$ transitions. Within this theoretical framework, differences between the MCD spectra of Co^{2+}Cbi$^+$ and the MetH^{CT}-bound Co^{2+}Cbl species can be interpreted in terms of molecular perturbations to the Co^{2+} coordination environment and/or the corrin π system induced by the enzyme active site.

The MCD spectra of I690C/G743C MetH^{CT}-bound Co^{2+}Cbl (Figure 2B) and Co^{2+}Cbi$^+$ (Figure 2A) are qualitatively very similar, indicating that the MetH^{CT} variant binds Co^{2+}Cbl in a

(22) Jarrett, J. T.; Goulding, C. W.; Fluhr, K.; Huang, S.; Matthews, R. G. *Methods Enzymol.* **1997**, *281*, 196–213.

(23) Nilges, M. J. Ph.D. Thesis, University of Illinois, Urbana-Champaign, 1979.

(24) Stich, T. A.; Yamanishi, M.; Banerjee, R.; Brunold, T. C. *J. Am. Chem. Soc.* **2005**, *127*, 7660–7661.

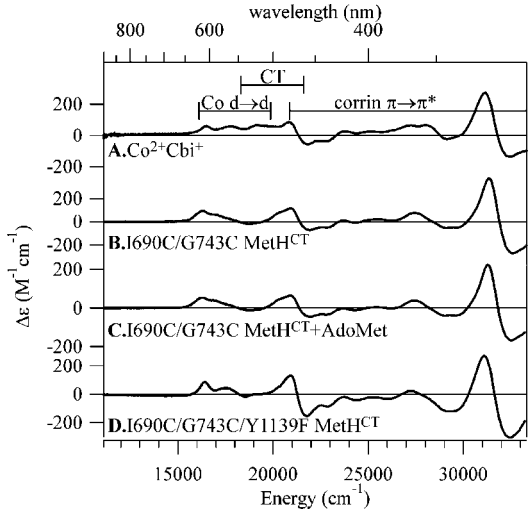


Figure 2. Low-temperature MCD spectra at 4.5 K and 7 T of (A) aqueous $Co^{2+}Cbi^{+}$, (B) I690C/G743C $MetH^{CT}$-bound $Co^{2+}Cbl$, (C) I690C/G743C $MetH^{CT}$-bound $Co^{2+}Cbl$ in the presence of excess AdoMet, and (D) I690C/G743C/Y1139F $MetH^{CT}$-bound $Co^{2+}Cbl$.

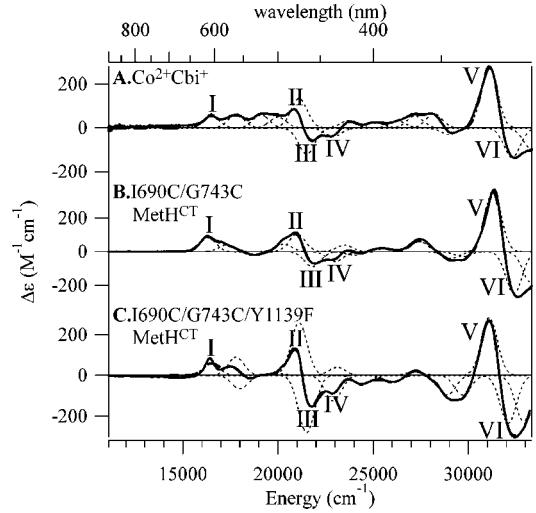


Figure 3. Solid lines: low-temperature MCD spectra at 4.5 K and 7 T of (A) aqueous $Co^{2+}Cbi^{+}$, (B) I690C/G743C $MetH^{CT}$-bound $Co^{2+}Cbl$, and (C) I690C/G743C/Y1139F $MetH^{CT}$-bound $Co^{2+}Cbl$. Dotted lines: Gaussian deconvolutions of the experimental spectra. Dashed lines: sum of individual Gaussian bands. The fit parameters for bands I−VI are provided in Table 1.

five-coordinate His-off form with a water molecule in the axial position. This finding lends support to the proposed coordination state of $Co^{2+}Cbl$ bound to full-length wild-type MetH in the activation conformation.[25] Interestingly, however, closer inspection of the two spectra in parts A and B of Figure 2 reveals some intriguing differences in the Co 3d → 3d region and the position of the intense derivative-shaped feature near 31 000 cm^{-1}. These subtle differences suggest that the $MetH^{CT}$ active site slightly perturbs the Co^{2+} coordination environment and/or the corrin π system from the relaxed structure adopted by $Co^{2+}Cbi^{+}$ in aqueous solution.

The effect of the AdoMet substrate on the MCD spectrum of I690C/G743C $MetH^{CT}$-bound $Co^{2+}Cbl$ was determined by adding a 3-fold excess of AdoMet (Figure 2B,C). As the MCD spectrum remained virtually unchanged in this process, it can be concluded that the presence of AdoMet in the enzyme active site does not perturb the geometric and electronic structures of $Co^{2+}Cbl$ bound to I690C/G743C $MetH^{CT}$ any further, at least under these sample conditions.

Because in the activation conformation the phenolic oxygen of Y1139 is located within 4.5 Å of the Co^{2+} center,[10] the MCD spectra of $Co^{2+}Cbl$ bound to I690C/G743C $MetH^{CT}$ and I690C/G743C/Y1139F $MetH^{CT}$ were compared to assess the effect of residue 1139 on the cofactor environment (Figure 2B,D). The two MCD spectra are qualitatively very similar, indicating that in each species $Co^{2+}Cbl$ is five-coordinate with an axially coordinated solvent molecule. However, small differences are evident in the Co 3d → 3d region and the position of the intense derivative-shaped feature near 31 000 cm^{-1}. Indeed, the MCD spectrum of I690C/G743C/Y1139F $MetH^{CT}$-bound $Co^{2+}Cbl$ is actually much more similar to that of $Co^{2+}Cbi^{+}$ (Figure 2A) than the I690C/G743C $MetH^{CT}$-bound $Co^{2+}Cbl$ spectrum. This result suggests that the Y1139F mutation essentially offsets the geometric and electronic structural perturbations that the I690C/G743C $MetH^{CT}$ active site imposes on its His-off bound $Co^{2+}Cbl$ cofactor. A quantitative analysis that correlates these MCD spectral changes with specific Y1139- and F1139-induced structural perturbations to the enzyme-bound $Co^{2+}Cbl$ is presented next.

(25) Bandarian, V.; Matthews, R. G. *Methods Enzymol.* **2004**, *380*, 152–169.

Table 1. Energies and Band Shifts from Gaussian Deconvolutions of the MCD Spectra Presented in Figure 3

	peak positions (cm^{-1})			band shifts (cm^{-1})	
band	$Co^{2+}Cbi^{+}$	Y1139	F1139	Y1139	F1139
I	16 500	16 300	16 500	−200	0
II	21 100	21 000	21 100	−100	0
III	21 500	21 800	21 500	300	0
IV	22 900	23 000	22 900	100	0
V	31 100	31 300	31 100	200	0
VI	32 300	32 400	32 100	100	−200

3.2. The Y1139 Residue Is Responsible for a Long Co−OH_2 Bond in $MetH^{CT}$-Bound $Co^{2+}Cbl$. Our MCD spectra presented in Figure 2 were quantitatively analyzed to identify key geometric and electronic structural perturbations of the $Co^{2+}Cbl$ cofactor induced by the $MetH^{CT}$ active site. First, the MCD spectra of $Co^{2+}Cbi^{+}$ and of $Co^{2+}Cbl$ bound to the $MetH^{CT}$ double and triple mutants were fit with 16 Gaussian bands of uniform width to deconvolute the experimental data into individual electronic transitions (Figure 3). Six of these sixteen bands had obvious counterparts in all three spectra. The observed band shifts for these six bands in the MCD spectra of $Co^{2+}Cbl$ bound to the two $MetH^{CT}$ variants from their positions in the $Co^{2+}Cbi^{+}$ spectrum are provided in Table 1. This analysis reveals that three of the six bands undergo significant shifts (>100 cm^{-1}; see the Materials and Methods for details) between the MCD spectra of $Co^{2+}Cbi^{+}$ and I690C/G743C $MetH^{CT}$-bound $Co^{2+}Cbl$ (Table 1). Band I was previously assigned to a Co $3d_{x^2-y^2} \rightarrow 3d_{z^2}$ electronic transition, while bands III and V were attributed to corrin $\pi \rightarrow \pi^*$ transitions.[18] Collectively, these band shifts indicate that the $MetH^{CT}$ active site perturbs the energies of both the Co 3d-based and the corrin π-based molecular orbitals (MOs). In contrast, only band VI, which was previously assigned to a corrin $\pi \rightarrow \pi^*$ transition, shifts by more than 100 cm^{-1} between the MCD spectra of $Co^{2+}Cbi^{+}$ and I690C/G743C/Y1139F $MetH^{CT}$-bound $Co^{2+}Cbl$. Therefore, our MCD data indicate that the active site of the $MetH^{CT}$ triple mutant does not affect the relative energies of the Co 3d-based

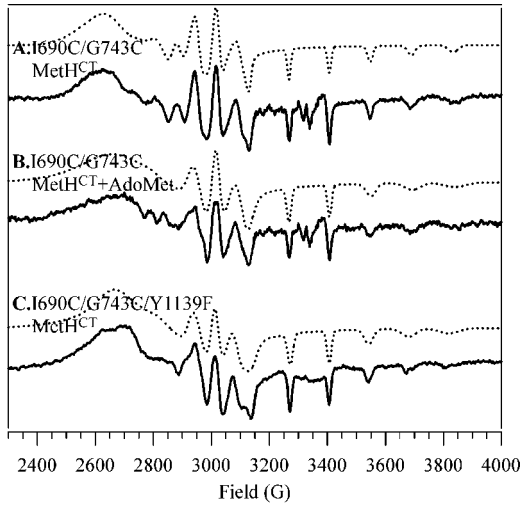


Figure 4. Solid lines: low-temperature X-band EPR spectra at 20 K of (A) I690C/G743C MetH^{CT}-bound Co^{2+}Cbl, (B) I690C/G743C MetH^{CT}-bound Co^{2+}Cbl in the presence of excess AdoMet, and (C) I690C/G743C/Y1139F MetH^{CT}-bound Co^{2+}Cbl. Dotted lines: simulations of the experimental spectra.

MOs of the His-off bound Co^{2+}Cbl cofactor, though it does slightly alter the electronic structure of the corrin π-system.

The observed MCD band shifts were analyzed within the framework of a spectroscopic/structural correlation for $Co^{2+}Cbi^{+}$, which was developed previously by using density functional theory computations to evaluate the effect of a systematic lengthening of the Co−OH_2 bond on the energies of the electronic transitions.[19] These calculations revealed that a lengthening of the Co−OH_2 bond causes a red shift of the Co $3d_{x^2-y^2} \rightarrow 3d_{z^2}$ transition (band I) and a blue shift of the lowest energy corrin $\pi \rightarrow \pi^*$ transition (band III). Although this previous study did not investigate the effect of the Co−OH_2 bond length on the positions of bands V and VI, the shifts observed for bands I and III from $Co^{2+}Cbi^{+}$ to I690C/G743C MetH^{CT}-bound Co^{2+}Cbl are consistent with a longer Co−OH_2 bond in the latter species. Similarly, the lack of any discernible shifts of band I or III between the MCD spectra of I690C/G743C/Y1139F MetH^{CT}-bound Co^{2+}Cbl and $Co^{2+}Cbi^{+}$ indicates that the axial Co−OH_2 distance is virtually identical in these two species.

3.3. EPR Spectroscopic Data Confirm That the Y1139 Residue Elongates the Co−OH_2 Bond of MetH^{CT}-Bound Co^{2+}Cbl. EPR spectroscopic studies of the MetH^{CT}-bound Co^{2+}Cbl species were conducted to corroborate the conclusions drawn from our MCD spectral analysis, as this technique provides a uniquely sensitive probe of the Co $3d_{z^2}$-based singly occupied MO (SOMO) that is oriented along the Co−OH_2 bond vector (Figure 4). All three spectra are qualitatively similar to one another and closely resemble that of $Co^{2+}Cbi^{+}$,[26] thus confirming that Co^{2+}Cbl is in the His-off form with an axially bound H_2O ligand when bound to both MetH^{CT} variants with or without AdoMet present.

The EPR spectra were fit assuming collinear **g** and **A** tensors to extract quantitative information about the axial coordination environment of Co^{2+}Cbl in these species (Figure 4 and Table 2). These fits revealed that the *g* values and $A(^{59}Co)$ hyperfine coupling constants for I690C/G743C MetH^{CT}-bound Co^{2+}Cbl

Table 2. Fit Parameters from Simulations of the EPR Spectra Presented in Figure 4

				^{59}Co *A* (MHz)		
	g_1	g_2	g_3	A_1	A_2	A_3
Y1139	2.406	2.320	1.995	231	208	387
+AdoMet	2.405	2.320	1.995	232	209	394
F1139	2.397	2.314	1.995	218	192	378

are virtually unchanged upon the addition of AdoMet, in agreement with the MCD spectra of these two species. In contrast, small differences exist between the EPR fit parameters for the two MetH^{CT} species, implying that the Y1139F mutation slightly alters the electronic structure of the active-site-bound Co^{2+}Cbl. Specifically, the deviations of the *g* values from their free electron value of 2.002 (i.e., *g* shifts) and the magnitudes of the $A(^{59}Co)$ values are uniformly larger for Co^{2+}Cbl bound to I690C/G743C MetH^{CT} than for the triple mutant. The larger *g* shifts observed for I690C/G743C MetH^{CT} suggest that the spin−orbit coupling between the ground state and the Co $3d_{xz,yz} \rightarrow 3d_{z^2}$ excited states is greater in this species, presumably due to a smaller ligand-field splitting of the Co 3d orbitals. Alternatively, the increased $A(^{59}Co)$ values indicate that in I690C/G743C MetH^{CT} the Co $3d_{z^2}$-based SOMO is more localized on the Co^{2+} ion and thus less delocalized onto the axially bound water ligand.

According to the previously developed spectroscopic/structural correlation for $Co^{2+}Cbi^{+}$,[19] a lengthening of the Co−OH_2 bond causes an increase in both the *g* shifts and the magnitude of the $A(^{59}Co)$ values. These changes can be rationalized in terms of a preferential stabilization of the Co $3d_{z^2}$-based SOMO relative to the filled Co 3d-based orbitals and an increased localization of the SOMO on the Co center as the axial Co−OH_2 is lengthened. Collectively, our MCD and EPR analyses provide compelling evidence for a longer Co−OH_2 bond of the enzyme-bound Co^{2+}Cbl species in I690C/G743C MetH^{CT} than in the MetH^{CT} triple mutant.

3.4. The Addition of AdoMet Substrate to MetH^{CT} Further Lengthens the Axial Co^{2+}−OH_2 Bond of the Cofactor at Physiologically Relevant Temperatures. Abs spectra were collected for enzyme samples in 10 mM KP_i buffer at 310 K to evaluate the effects of the Y1139 active-site residue and the AdoMet substrate on the geometric and electronic properties of MetH^{CT}-bound Co^{2+}Cbl under typical assay conditions.[25] As shown in Figure 5, the addition of AdoMet to I690C/G743C MetH^{CT}-bound Co^{2+}Cbl at 310 K causes a blue shift of the dominant Abs feature in the visible region by 2.5 nm. This blue shift was not observed at 4.5 K, which strongly suggests that the perturbation to the Co^{2+}Cbl structure triggered by the addition of AdoMet is entropy driven (possibly involving a slight change in the protein structure in the vicinity of the active site).[27] Alternatively, the Y1139F mutation causes a small red shift of this feature by 1 nm, suggesting that the differences between the MetH^{CT} double and triple mutants observed by MCD and EPR spectroscopies persist at 310 K.

The dominant feature in the visible region of the Abs spectra of Co^{2+} corrinoids has been shown to possess contributions from several electronic transitions, with the primary contributor

(26) Van Doorslaer, S.; Jeschke, G.; Epel, B.; Goldfarb, D.; Eichel, R.-A.; Kraütler, B.; Schweiger, A. *J. Am. Chem. Soc.* **2003**, *125*, 5915–5927.

(27) The lack of any discernible shift upon the addition of AdoMet at low temperature is not solely due to the presence of glycerol, the glassing agent used in our low-temperature MCD and Abs studies, because the EPR fit parameters of the MetH^{CT} double mutant are also unchanged upon the addition of AdoMet.

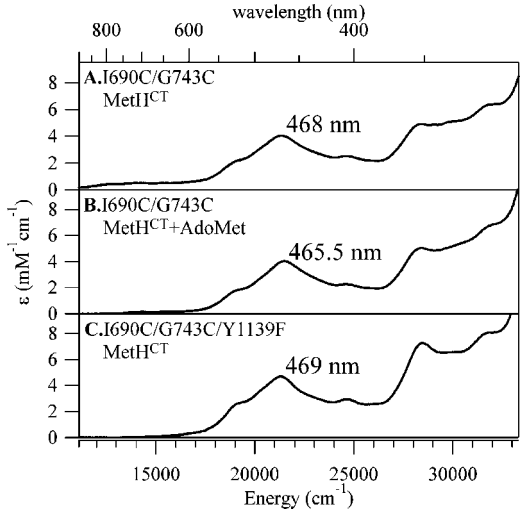


Figure 5. Abs spectra of (A) I690C/G743C MetH^{CT}-bound Co^{2+}Cbl, (B) I690C/G743C MetH^{CT}-bound Co^{2+}Cbl in the presence of excess AdoMet, and (C) I690C/G743C/Y1139F MetH^{CT}-bound Co^{2+}Cbl obtained at 310 K.

corresponding to the lowest energy corrin $\pi \rightarrow \pi^*$ transition (band III).[18] A computational study of $Co^{2+}Cbi^+$ performed previously revealed that a blue shift of band III is indicative of a lengthening of the axial $Co-OH_2$ bond.[19] Hence, our Abs data are consistent with a longer $Co-OH_2$ bond of Co^{2+}Cbl in I690C/G743C MetH^{CT} than in the triple mutant (Figure 5A,C) or in $Co^{2+}Cbi^+$.[28] Similarly, the 2.5 nm blue shift observed in the Abs spectrum of Co^{2+}Cbl bound to I690C/G743C MetH^{CT} upon the addition of AdoMet can thus be interpreted as an additional elongation of the $Co-OH_2$ bond in the ternary complex at 310 K. Collectively, the 4.5 K MCD, 20 K EPR, and 310 K Abs spectra of Co^{2+}Cbl bound to the two MetH^{CT} variants investigated here indicate that, under physiologically relevant conditions, both the Y1139 active site residue and the AdoMet substrate cause a lengthening of the axial $Co-OH_2$ bond of the enzyme-bound cofactor relative to that of free $Co^{2+}Cbi^+$.

3.5. MCD and Abs Spectroscopic Data Indicate That MetH^{CT}-Bound MeCbl Is a Five-Coordinate Species. A stopped-flow Abs spectroscopic study led to the proposal that the initial product of methyl transfer to Co^{1+}Cbl during the MetH activation process is a His-off bound MeCbl species that subsequently converts to the His-on form involved in Met biosynthesis.[9] A useful model for the His-off MeCbl species initially formed in this process is provided by $MeCbi^+$, an MeCbl derivative that lacks the nucleotide loop and possesses a water molecule in the axial coordination site previously occupied by DMB.[17]

The 310 K MCD and Abs spectra of $MeCbi^+$ and I690C/G743C MetH^{CT}-bound MeCbl are shown in Figures 6 and 7.[29] A comparison of the two data sets reveals substantial differences in the spectral region below 25 000 cm^{-1} (above 400 nm), which is dominated by corrin $\pi \rightarrow \pi^*$ transitions and Co 3d $\rightarrow \pi^*$ CT transitions.[17] Our data therefore suggest that the MetH^{CT} active site considerably perturbs the Co^{3+} coordination environment

(28) Jarrett, J. T.; Amaratunga, M.; Drennan, C. L.; Scholten, J. D.; Sands, R. H.; Ludwig, M. L.; Matthews, R. G. *Biochemistry* **1996**, *35*, 2464–2475.

(29) I690C/G743C MetH^{CT}-bound MeCbl was recently investigated by Abs spectroscopy under typical assay conditions for MetH (i.e., 10 mM KP_i buffer at 37 °C).[14] The published Abs spectrum is nearly identical to the one presented in this work.

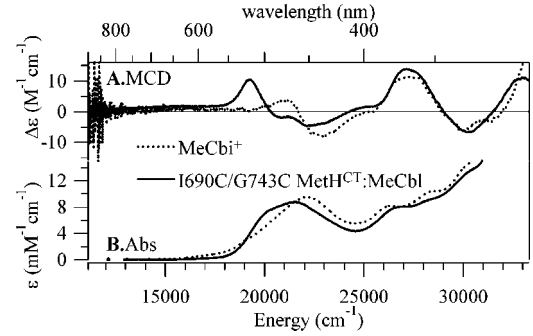


Figure 6. (A) MCD spectra at 7 T and (B) Abs spectra of aqueous $MeCbi^+$ (solid lines) and I690C/G743C MetH^{CT}-bound MeCbl (dotted lines) obtained at 310 K.

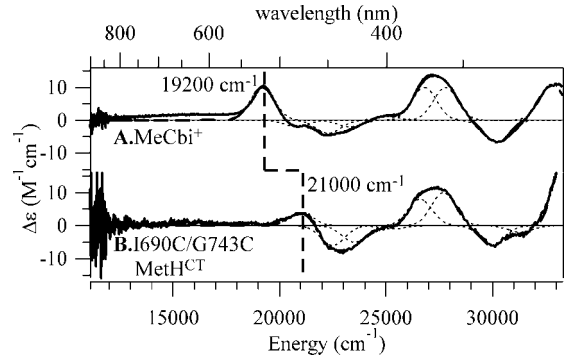


Figure 7. Solid lines: MCD spectra at 7 T of (A) aqueous $MeCbi^+$ and (B) I690C/G743C MetH^{CT}-bound MeCbl obtained at 310 K. Dotted lines: Gaussian deconvolutions of the experimental spectra. Dashed lines: sum of individual Gaussian bands.

and/or the corrin π-system from the relaxed structure found in free $MeCbi^+$.

The MCD spectra of $MeCbi^+$ and I690C/G743C MetH^{CT}-bound MeCbl were fit with nine Gaussian bands of uniform width to determine the energies of the individual electronic transitions contributing to the experimental spectra and explore the exact nature of this structural perturbation (Figure 6). The largest difference between the enzyme-bound species and free $MeCbi^+$ is an 1800 cm^{-1} blue shift of the lowest energy transition in the former spectrum. A similarly blue-shifted low-energy MCD feature was previously observed for MeCbl bound to H759G MetH, where it was shown to serve as a spectroscopic signature of a five-coordinate MeCbl species that lacks any significant axial bonding interaction trans to the $Co-C(H_3)$ bond.[16] This change in coordination number from six to five leads to a preferential stabilization of the donor MO involved in the lowest energy electronic transition and, thus, a blue shift of the corresponding MCD feature. Consequently, our MCD data provide compelling evidence that I690C/G743C MetH^{CT}-bound MeCbl is a five-coordinate species, which strongly suggests that the cofactor also adopts this unusual coordination number in the activation conformation of wild-type MetH.

4. Discussion

Although the molecular mechanism of the MetH activation process has been the subject of several studies,[9,30,31] at least

(30) Banerjee, R. V.; Harder, S. R.; Ragsdale, S. W.; Matthews, R. G. *Biochemistry* **1990**, *29*, 1129–1135.

two fundamental questions remain unanswered. First, it is not known how MetH catalyzes the thermodynamically challenging reduction of Co^{2+}Cbl to Co^{1+}Cbl by Fld. Previous studies have established that MetH-bound Co^{2+}Cbl is a five-coordinate, His-off species with an axially coordinated water molecule.[9] However, substitution of the His ligand by a water molecule is not sufficient for MetH to accomplish $Co^{2+}Cbl \rightarrow Co^{1+}Cbl$ reduction on the basis of the reduction potentials of the oxidized/semiquinone and semiquinone/hydroquinone couples of Fld (−260 and −440 mV, respectively, vs SHE in 10 mM KP_i buffer, pH 7.2)[31] and $Co^{2+}Cbi^+$ (−490 mV vs SHE at pH 9).[32] A second open question is when the $Co-N_{H759}$ bond formation in the MeCbl product occurs relative to the conformational conversion of MetH into a catalytic conformation. Below, the implications of the spectroscopic data obtained in this study with respect to these two fundamental questions are discussed.

4.1. MetH Destabilizes His-Off Co^{2+}Cbl via a Hydrogen Bond Interaction. The MCD, EPR, and Abs spectroscopic results presented in this work indicate that the Y1139 residue perturbs the electronic structure of MetH-bound Co^{2+}Cbl. The estimated distance between the phenolic oxygen of this residue and the O atom of the axial water ligand of the cofactor is 2.3 Å, on the basis of the relative positions of the Co center and the Y1139 side chain in the X-ray crystal structure of I690C/G743C $MetH^{CT}$ and the $Co-OH_2$ bond length of 2.22 Å in $Co^{2+}Cbi^+$, as determined by X-ray absorption spectroscopy.[14,33] Consequently, a hydrogen bond interaction likely exists between the Y1139 residue and the axial water ligand. Indeed, our 4.5 K MCD (Figure 3 and Table 1), 20 K EPR (Figure 4 and Table 2), and 310 K Abs (Figure 5) spectroscopic data, interpreted within the framework of spectroscopic/structural correlations established for $Co^{2+}Cbi^+$,[19] indicate that this hydrogen bond interaction does exist and serves to lengthen the $Co-OH_2$ bond of the cofactor by ~0.05 Å, thereby causing a stabilization of the Co $3d_{z^2}$-based "redox-active" MO and, thus, an increase in the $Co^{2+/1+}$ reduction potential by about 50 mV.[34] Remarkably, this predicted value corresponds precisely to the experimental difference in the $Co^{2+}Cbl/Co^{1+}Cbl$ couple of the cofactor bound to the I690C/G743C and I690C/G743C/Y1139F $MetH^{CT}$ mutants, as determined in preliminary electrochemical measurements.

The strategy apparently utilized by MetH to activate Co^{2+}Cbl for $Co^{2+} \rightarrow Co^{1+}$ reduction is more subtle than the one employed by the adenosyltransferase (ATR) enzymes that catalyze the Co−C(Ado) bond formation step in the biosynthesis of coenzyme B_{12}. Spectroscopic and X-ray crystallographic studies revealed that ATR enzymes promote the formation of a four-coordinate Co^{2+}Cbl intermediate in the presence of the cosubstrate adenosine-5′-triphosphate.[19,24] This coordination number change has been estimated to raise the reduction potential of Co^{2+}Cbl by an additional ~250 mV, to about −240 mV, and thus into a range accessible to physiological reductants,[19] yet activation of Co^{2+}Cbl via axial bond elongation or cleavage is not the only means by which enzymes accomplish the thermodynamically challenging $Co^{2+}Cbl \rightarrow Co^{1+}Cbl$ reduction. For example, the corrinoid/iron−sulfur protein (CFeSP), whose physiological role is to transfer a methyl group to the active-site A-cluster of acetyl-CoA synthase, utilizes a low-potential Fe_4S_4 cluster ($E° = -523$ mV vs SHE)[35] to reduce a five-coordinate, His-off bound Co^{2+}Cbl species with an axially coordinated water molecule in its activation cycle.[20] In this case, electron transfer from the $[Fe_4S_4]^+$ cluster to CFeSP-bound Co^{2+}Cbl ($E° = -504$ mV vs SHE)[35] is thermodynamically favorable, by ~20 mV. It is therefore not surprising that the Y1139 active-site residue of MetH, which serves to weaken the $Co-OH_2$ bond of the enzyme-bound Co^{2+}Cbl species, has no counterpart in CFeSP.[36] Consistent with these findings, the MCD spectrum of CFeSP-bound Co^{2+}Cbl provides clear evidence against a lengthened $Co-OH_2$ bond. In summary, while ATR enzymes and the CFeSP catalyze the reduction of enzyme-bound Co^{2+}Cbl by forming a four-coordinate Co^{2+} center and using a low-potential Fe_4S_4 cluster, respectively, MetH utilizes a hydrogen bond interaction to lengthen the axial $Co-OH_2$ bond of the cofactor.

4.2. AdoMet Promotes a Stronger Hydrogen Bond Interaction between Y1139 and MetH-Bound Co^{2+}Cbl. Our Abs spectroscopic data presented in this work (Figure 5) suggest that AdoMet is responsible for an additional increase in the $Co^{2+/1+}$ reduction potential of MetH-bound Co^{2+}Cbl. X-ray crystallographic studies revealed that the AdoMet substrate binds to MetH in a pocket located directly behind Y1139, from the point of view of Cbl.[10] It is reasonable to assume that binding of AdoMet to MetH pushes Y1139 away from this binding pocket and toward MetH-bound Co^{2+}Cbl. The closer proximity of Y1139 and MetH-bound Co^{2+}Cbl should favor the formation of a stronger hydrogen bond interaction between the phenolic group of Y1139 and the axial water ligand bound to the β-face of the cofactor, thus causing an additional lengthening of the $Co-OH_2$ bond. This scenario is consistent with our 310 K Abs spectra, which show the changes expected for a $Co-OH_2$ bond elongation of MetH-bound Co^{2+}Cbl in response to AdoMet binding. A longer $Co-OH_2$ bond directly correlates with an elevated reduction potential of MetH-bound Co^{2+}Cbl, because this axial bond elongation stabilizes the Co $3d_{z^2}$-based redox-active MO (vide supra).

The extent by which the $Co-OH_2$ bond of Co^{2+}Cbl is lengthened upon AdoMet binding to I690C/G743C $MetH^{CT}$ can be assessed on the basis of the 2.5 nm blue shift of the dominant feature in the visible region of the Abs spectrum (Figure 5). A similar blue shift of this feature was observed in our recent study of Co^{2+}Cbl bound to H759G MetH,[16] where complementary EPR and MCD spectroscopic experiments revealed that this band shift reflects the formation of a four-coordinate, square-planar Co^{2+}Cbl species.[16] Therefore, on the basis of the results obtained in this study, it appears that the Y1139 active-site residue and the AdoMet substrate work in concert to generate an effectively four-coordinate form of MetH-bound Co^{2+}Cbl at physiologically relevant temperatures. Indeed, a preliminary X-ray crystal structure of I690C/G743C $MetH^{CT}$ in the Co^{2+}Cbl state and with *S*-adenosylhomocysteine bound to the active site confirms that the Co^{2+} center is in an essentially square-planar, four-coordinate ligand environment, with a water molecule positioned closer to the Y1139 residue (3.3 Å) than the cobalt ion (3.6 Å) (M. Koutmos and R. Matthews, unpublished results). The fact that this "activated" Co^{2+}Cbl species is only formed in the presence of the AdoMet substrate suggests that the

(31) Hoover, D. M.; Jarrett, J. T.; Sands, R. H.; Dunham, W. R.; Ludwig, M. L.; Matthews, R. G. *Biochemistry* **1997**, *36*, 127–138.
(32) Lexa, D.; Saveant, J. M.; Zickler, J. *J. Am. Chem. Soc.* **1980**, *102*, 4851–4852.
(33) Giorgetti, M.; Ascone, I.; Berrettoni, M.; Conti, P.; Zamponi, S.; Marassi, R. *J. Biol. Inorg. Chem.* **2000**, *5*, 156–166.
(34) Presumably, Y1139 acts a hydrogen bond donor. This makes the H_2O ligand a weaker σ-bond donor to Co^{2+} and lengthens the Co−O bond.
(35) Harder, S. R.; Lu, W.-P.; Feinberg, B. A.; Ragsdale, S. W. *Biochemistry* **1989**, *28*, 9080–9087.
(36) Svetlitchnaia, T.; Svetlitchnyi, V.; Meyer, O.; Dobbek, H. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 14331–14336.

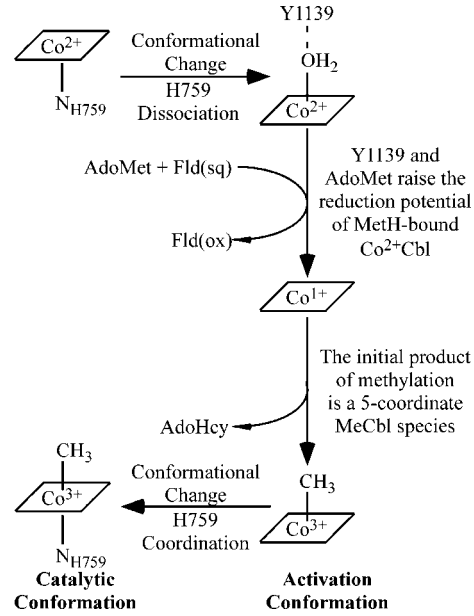
enzyme exerts significant control over the timing of the Co^{2+}Cbl $\rightarrow$ Co^{1+}Cbl reduction, to suppress the likelihood of deleterious side reactions involving the transiently formed Co^{1+}Cbl "super-nucleophile".

4.3. MetH-Bound MeCbl Is a Five-Coordinate Species in the Activation Conformation. Our Abs and MCD spectra of I690C/G743C $MetH^{CT}$-bound MeCbl reveal that a five-coordinate form of MeCbl is bound to MetH in the activation conformation (Figures 5 and 6). This five-coordinate species must be transformed to a six-coordinate, His-on MeCbl form, and the enzyme has to undergo a conformational conversion from the activation conformation into a catalytic conformation to complete the activation process.[9,12,13] Since $Co-N_{H759}$ bond formation does not occur in the MetH activation conformation, this bond must be formed concomitant with, or subsequent to, the conformational conversion of MetH into a catalytic conformation. This finding is consistent with a recent X-ray crystallographic study of I690C/G743C $MetH^{CT}$, which revealed the presence of a salt bridge between $H759^{+}$ and $D1093^{-}$.[14] Located in the activation module, D1093 is a highly conserved residue among all MetH homologues. As this salt bridge cannot exist in the catalytic conformations, where the Cbl-binding module and the activation modules are separated, H759 is free to bind to the Co center and form the six-coordinate MeCbl species involved in Met biosynthesis.

5. Summary and Conclusions

Scheme 1 summarizes the mechanistic implications of the spectroscopic studies presented in this work. The conformational conversion of MetH into the activation conformation breaks the axial $Co-N_{H759}$ bond in MetH-bound Co^{2+}Cbl. A hydrogen bond interaction between the resulting five-coordinate, His-off form of MetH-bound Co^{2+}Cbl and Y1139 stabilizes the Co $3d_{z^2}$-based redox-active MO by elongating the axial $Co-OH_2$ bond. The addition of AdoMet enhances this interaction between Y1139 and MetH-bound Co^{2+}Cbl, causing a partial dissociation of the axially coordinated water molecule, thus likely raising the $Co^{2+/1+}$ reduction potential into a range accessible to the physiological reducing agent Fld. The Co^{1+}Cbl intermediate produced by reduction of MetH-bound Co^{2+}Cbl with Fld is rapidly methylated by AdoMet to yield a five-coordinate, His-off form of MeCbl.[9] This five-coordinate species is transformed to a six-coordinate, His-on form of MeCbl concomitant with, or subsequent to, the conformational conversion of MetH into a catalytic conformation.

Scheme 1



Acknowledgment. This work was supported by the National Science Foundation (CAREER Grant MCB-0238530 to T.C.B.) and the National Institutes of Health (Grant GM29408 to R.G.M.). We acknowledge Dr. Angela Fleischhacker and Dr. Jeanne Sirovatka for the preparation of the $MeCbi^{+}$ MCD sample and thank them as well as Dr. Markos Koutmos for stimulating discussions about MetH. We also thank Prof. Mark Nilges and the Illinois EPR Research Center for providing us with a copy of the SIMPOW6 program.

Supporting Information Available: Abs (4.5 K) data of $MetH^{CT}$-bound Co^{2+}Cbl and complete parameter sets for the Gaussian deconvolutions of the MCD data and the SIMPOW6 simulations of the EPR data. This material is available free of charge via the Internet at http://pubs.acs.org.

JA8038129