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Mercury Methylation by Cobalt Corrinoids: Relativistic Effects Dictate the Reaction Mechanism

Taye B. Demissie,¹ Brady D. Garabato,² Kenneth Ruud,¹ and Pawel M. Kozlowski^{2,3,*}

Abstract: The methylation of $\text{Hg(II)(SCH}_3)_2$ by corrinoid-based methyl donors proceeds in a concerted manner through a single transition state by transfer of a methyl radical, in contrast to previously proposed reaction mechanisms. This reaction mechanism is a consequence of relativistic effects that lower the energies of the mercury $6p_{1/2}$ and $6p_{3/2}$ orbitals, making them energetically accessible for chemical bonding. In the absence of relativistic effects, the predicted reaction mechanism is qualitatively different. This is the first example of relativity being decisive for the nature of the observed reaction mechanism.

Methylation is the process of controlled transfer of a methyl group onto amino acids, proteins, enzymes, DNA, or other biological intermediates. This process is one of the essential metabolic functions, and is catalyzed by a variety of enzymes including corrinoid-dependent methyltransferases (MeTrs).^[1-3] One of the most well-studied MeTrs is cobalamin-dependent methionine synthase, which catalyzes the transfer of the methyl group of $\text{CH}_3\text{-H}_4\text{folate}$ to homocysteine to form methionine.^[4-5] Other MeTrs, such as $\text{CH}_3\text{-H}_4\text{folate:corrinoid-iron/sulfur}$, a module in the acetyl-CoA synthase,^[6,7] and methanol:coenzyme M have also been well studied.^[8]

Cobalt corrinoids (Fig. 1) can formally transfer a methyl group in the form of a carbocation (CH_3^+), a radical (CH_3^\cdot), or a carbanion (CH_3^-). Individual methyl transfer reactions have been traditionally interpreted as $\text{S}_\text{N}2$ -type displacements (i.e., formally involving CH_3^+).^[2-4] Alternative mechanisms based on reductive cleavage (or one-electron reduction) of the corrinoid induced by substrates have also been proposed.^[9-12] The key step in this reductive cleavage mechanism involves formation of a diradical species, which pre-activates the reactant complex and facilitates homolytic cleavage of the Co-C bond followed by CH_3^\cdot transfer. However, until recently there was no evidence for a methyl anion transfer, which would result in a Co(III) product (or intermediate) after Co-C bond heterolysis. The involvement of CH_3^- has been recently invoked, however, in the context of Hg(II) methylation, specifically in conjunction with bacterial mercury methylation (HgcA) from *D. desulfuricans ND132*. The feasibility of CH_3^- involvement in methyl transfer reactions was further supported by density functional theory (DFT) calculations of ligand exchange reactions (LERs) between cobalt-corrinoids and Hg(II)-based substrates.^[13]

Herein we demonstrate that relativistic effects are crucial

for a proper description of LERs between Hg(II) and corrinoid-based methyl donors (Fig. 1). It is shown that inclusion of spin-orbit coupling (SOC) effects changes the mechanism of the LER, which becomes more consistent with radical methyl transfer, rather than involving a methyl carbocation. Furthermore, the presence of SOC changes the nature of the reaction pathway, from a step-wise to a concerted LER between Co(III) and Hg(II).

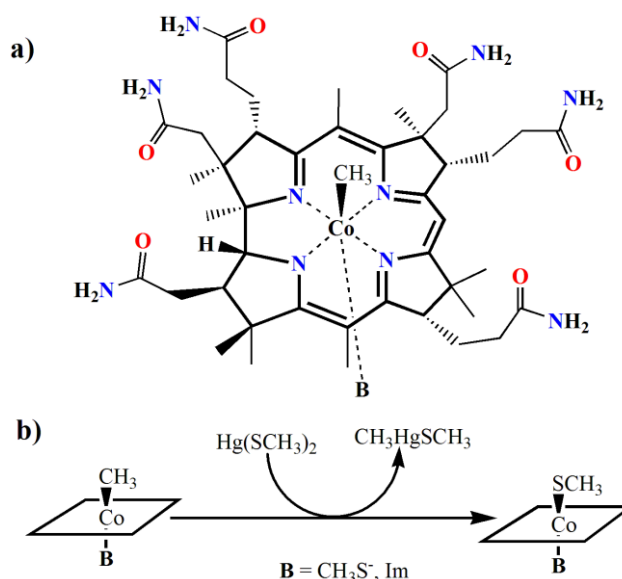


Figure 1. (a) Molecular structure of cobalt-corrinoid. (b) Ligand exchange reaction (LER) between $\text{Hg(II)(SCH}_3)_2$ and $\text{B-[Co(III)corrin]-CH}_3^+$.

In this study, we consider the LER of $\text{Hg(II)(SCH}_3)_2$ methylation by $\text{B-[Co(III)corrin]-CH}_3^+$ (Fig. 1b), resulting in $\text{CH}_3\text{Hg(II)SCH}_3$ and $\text{B-[Co(III)corrin]-SCH}_3^+$. As in previous computational studies,^[14-15] a truncated structure of the corrin was used (Fig. 1a). To obtain a reliable description of the LER, relativistic BP86-D3/DFT calculations employing the zeroth-order regular approximation (ZORA) to the Dirac equation were applied. In addition, SOC was included in the ZORA Hamiltonian (SO-ZORA), and relativistic basis sets for all atoms were used. Environmental effects were modelled via COSMO/ H_2O as implemented in the ADF suite of programs for electronic structure calculations (see Methods section for details).^[16]

Potential energy surfaces (PESs) computed at the SO-ZORA/BP86-D3/TZ2P (COSMO/ H_2O) level of theory are shown in Fig. 2 for the LERs, where imidazole (Im) and cysteine (Cys) are the lower-axial ligands coordinated to Co(III).

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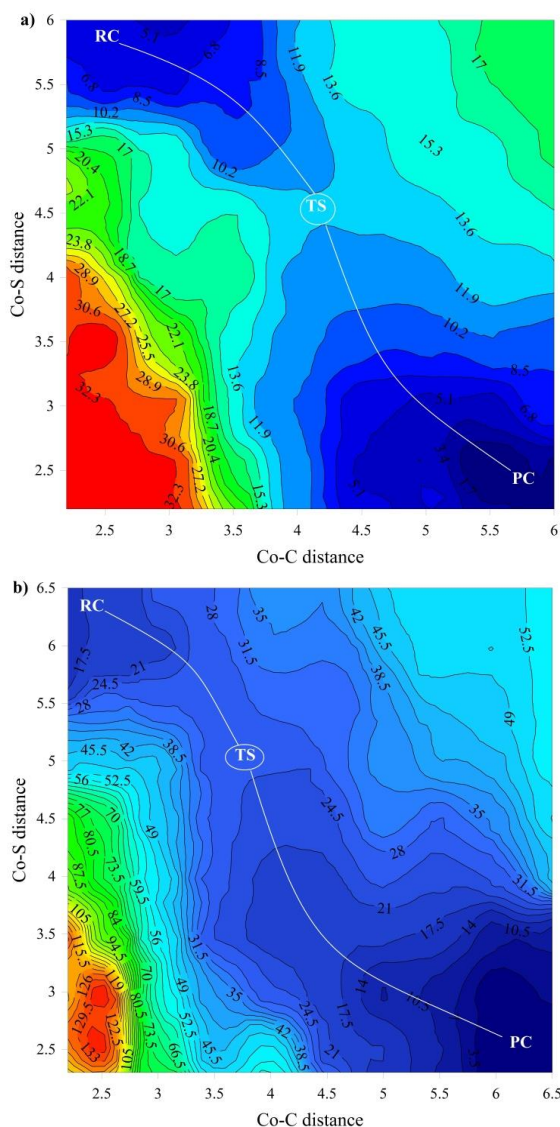


Figure 2. PESs corresponding to LERs employing the SO-ZORA/BP86-D3/TZ2P (COSMO/H₂O) level of theory. Upper map B = Im, lower B = Cys.

Although trans-nitrogenous ligands, such as dimethylbenzimidazole (DBI) and histidine (His) are commonly found in B₁₂-dependent enzymes, in the case of HgcA, a strictly conserved Cys residue was predicted as the lower Co(III) coordinated ligand.^[17] It was further argued that the presence of a Cys axial base in HgcA enhances the methylation of Hg(II). Both PESs were constructed as functions of Co-C and Co-S distances, and although other internal coordinates were tested, the most transparent description of the LERs was provided by PESs with coordinates associated with the Co(III) center (Fig. 2). Overall, regardless of axial base, relativistic DFT calculations give very consistent descriptions of the mechanisms associated with the LERs. The reactant complex (RC) is in the minimum reaction pathway connected to the product complex (PC) and proceeds via a single energy maximum (TS). Relativistic DFT calculations clearly predict a concerted, rather than a step-wise,

character for the ligand exchange, in contrast to previous suggestions.^[13]

Let us first consider the LERs between Co(III) and Hg(II) without relativistic effects. Using the same structural models and basis sets, the nonrelativistic (NR) PES was constructed based on BP86-D3/TZ2P (COSMO/H₂O) calculations (Fig. 3; note that only one surface is shown with B = Im, as different axial bases produce qualitatively similar PESs). The NR PES looks very different from the corresponding PESs shown in Fig. 2. Starting from the RC, a minimum energy path lies parallel to the Co-C coordinate, implying that the initial step involves cleavage of one bond without formation of the other. The energy minimum corresponding to the reaction intermediate (RI) involves formation of CH₃S⁻, and CH₃Hg(II)SCH₃. Furthermore, presence of a Co(III)-based corrinoid indicates that the Co-C bond is cleaved heterolytically, and that the methyl group is formally transferred in the LER as CH₃⁻.^[13] Thus, the absence of relativistic effects leads to a very different mechanistic picture of the LER, now proceeding in a step-wise manner involving CH₃⁻.

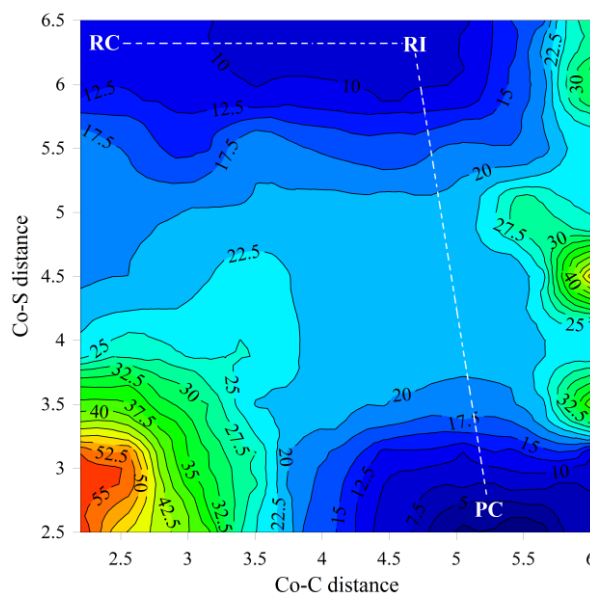


Figure 3. PES corresponding to ligand exchange reaction (B = Im) employing NR-BP86-D3/TZ2P (COSMO/H₂O) level of theory.

To further explore the nature of the LERs, the corresponding TSs were located and characterized including relativistic effects. For compatibility with the PESs shown in Fig. 2, the corresponding energy profiles were computed with respect to the RC (Fig. 4). In addition, Figure 4 summarizes spin density profiles, as well as key structural parameters. Regardless of axial base used in the SO-ZORA/BP86-D3/TZ2P (COSMO/H₂O) calculations, the predicted LERs are consistent with radical mechanisms. This conclusion is supported by an analysis of the spin density profiles, which show the unpaired electrons located either on Co and C, or S depending on the axial base, consistent with the open-shell diradical character of the TSs. The spin density associated with the cobalt center has a d₂₂ orbital shape, indicating a Co(II) character, further supporting that the LERs involve transfer of a methyl group radical.

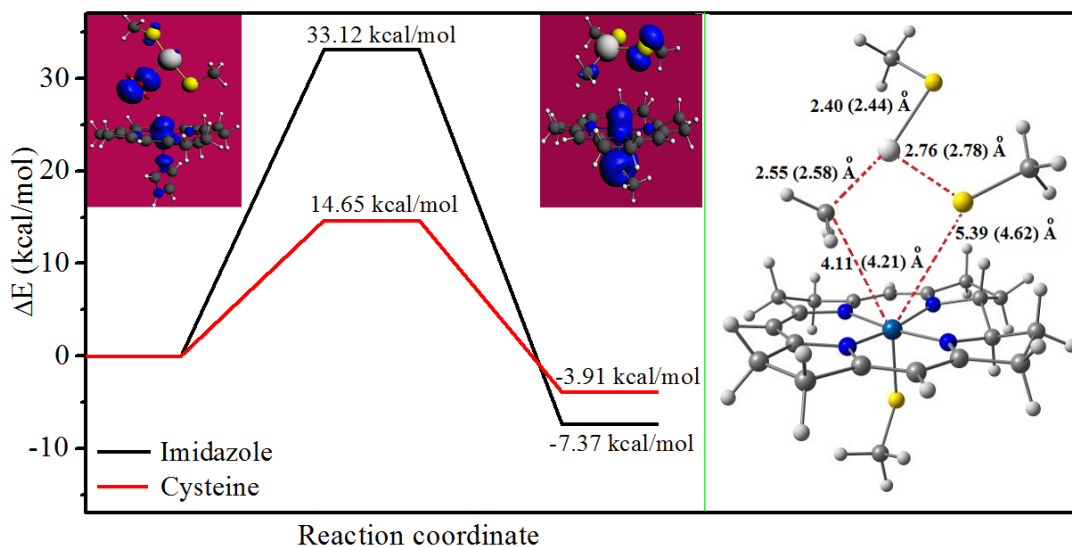


Figure 4. (Left) Reaction profiles along with spin densities of the LER of $\text{Hg(II)(SCH}_3)_2$ methylation by $\text{B-[Co(III)corrin]-CH}_3^+$ for B=Imidazole , and Cysteine . (Right) structure of the transition state corresponding to B=Cysteine . The insets (spin densities) correspond to B=imidazole (left corner) and B=cysteine (middle).

The key difference between the LERs where the axial base is Cys or Im is a noticeable energy difference between the RC and the TS, which in the case of Im is almost twice as large. These differences in energy can be attributed to the trans effect associated with the axial base, as can be inferred from the spin density profiles associated with the Im and Cys ligands (Fig. 4). In the case of Im, the majority of the spin is located on Co and C_{Me} , with only a small contribution associated with N_{Im} . The corresponding TS can thus be described as an early TS. On the other hand, the spin density distribution looks quite different for Cys. The spin density is largely shared between Co(II) and S_{Hg} , but with a non-negligible contribution on S_{Cys} . In contrast to Im, the corresponding TS can be viewed as a late TS, which is more close to the PC than the RC. The ability of S_{Cys} to accommodate more electron density in comparison to N_{Im} stabilizes the TS by shifting the spin density associated with the upper ligand from C_{Me} to S_{Me} .

Further understanding of the reaction mechanism, where the trans axial base is Cys, can be obtained from an analysis of the eigenvector associated with the imaginary frequency $i116\text{ cm}^{-1}$. The TS can be described as having no motion along the Co-C coordinate, which is consistent with a distance between Co and C_{Me} in the TS equal to 4.11 Å, compared to an equilibrium Co-C bond length of 2.00 Å. Likewise, the distance between C and S_{Me} of 5.39 Å is also long. However, the concerted nature of the reaction, in which the CH_3 group is partially transferred towards Hg(II) while the SCH_3 group is still weakly bound, should be stabilized by the presence of cob(II)alamin. This observation is in agreement with the hypothesis that cob(II)alamin acts as a conductor involved in stabilization of highly reactive product-related methylene radicals in B_{12} -dependent enzymatic catalysis.^[18]

It is interesting to understand why the LER mechanism obtained without relativistic effects is so different. The reason can be found in the electronic structure of Hg(II). When

including relativistic effects, the relative energy levels of the atomic orbitals change, and subshells having $l > 0$ split into two subgroups ("spinors") due to SOC.^[19, 20] Specifically, the 6s orbitals of Hg decrease substantially in energy, whereas the 6p orbitals decrease in energy to a lesser extent. The 6p subshell splits into $6p_{1/2}$ and $6p_{3/2}$ spinors. The energetic accessibility of the 6p orbitals is clearly enhanced in the coordination sphere of Hg(II) when the structure of the TS is taken into consideration (see Figs S1 and S2, SI). The ability of mercury to coordinate three ligands, CH_3 in addition to two SCH_3 groups in T-shaped complex (Fig. 4), is consistent with mixing of 6s and the two 6p orbitals. The frontier orbitals of the corresponding TS are presented in Figs. S3-S6 of the SI. Without SOC, the 6p orbitals are not energetically accessible and cannot participate in chemical bonding. Consequently, Hg(II) cannot accommodate three ligands, and SCH_3^- is released as an intermediate in order to accommodate the methyl group coordinated to mercury in the non-relativistic regime.

In conclusion, theory only supports a carbanion transfer to Hg(II) when SOC is omitted. When relativistic effects are taken into account, the TS has a multi-centered diradical character, where methyl transfer occurs in the form of a radical.

Methods

The computational methodologies are presented in the supporting information.

Acknowledgements

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