Mechanism of Cobalamin-Mediated Reductive Dehalogenation of Chloroethylenes

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Abstract

Reductive dehalogenation involving cobalamin has been proven to be a promising strategy for decontamination of polluted environments. However, cob(I)alamin can act both as a strong reductant and a powerful nucleophile, and thus several competing dehalogenation pathways may be involved. This work uses experimentally calibrated density functional theory on realistic cobalamin models to resolve controversies of cobalamin-mediated reduction of chloroethylenes by exploring mechanisms of electron transfer, nucleophilic substitution, and nucleophilic addition. The computational results provides molecular-level insight into the competing pathways for chloroethylenes reacting with cob(I)alamin: the computed ratios of inner-sphere to outer-sphere pathways for perchloroethylene and trichloroethylene are 17:1 and 3.5:1 respectively in accord with corresponding experimental ratios of $> 10:1$ and $> 2.3:1$, while the computed outer-sphere pathway for other less-chlorinated ethylenes is hampered by high barriers ($> 25$ kcal/mol). Thus, a new mechanistic picture has been obtained that the highly-chlorinated ethylenes primarily react via an inner-sphere nucleophilic-substitution pathway, while the less-chlorinated ethylenes mainly react through an inner-sphere nucleophilic-addition pathway. Especially, the quantitative comparison of standard reduction potentials between of the formed chlorinated cobalamin and cob(II)alamin/cob(I)alamin couple can be used to distinguish whether the inner-sphere pathway can proceed or not, and the linear free energy relationships have been developed to predict the reductive dehalogenation reactivity within a given mechanism. Furthermore, we have proposed new dual isotope analyses for distinguishing the various environmental dehalogenation mechanisms.
Introduction

The widespread industrial application of halogenated compounds as solvents, chemical intermediates and pesticides are of great environmental concern due to the adverse effects on ecosystems and human health.\textsuperscript{1,2} The negative effects of halogenated compounds are generally attributed to the halogen atoms; thus, the transformation of halogenated compounds to less- or non-halogenated products is a promising remediation strategy.\textsuperscript{3,4} Among various dehalogenation remediation strategies, cobalamin (vitamin B12) promoted dehalogenation reactions have garnered considerable attention, owing to evidence that the cell component responsible for dehalogenation reactions by several anaerobic bacteria is most likely this transition-metal coenzyme.\textsuperscript{5}

Cobalamin is the largest by molecular mass and arguably the most complex (in terms of functional groups) cofactor in biology, consisting of a cobalt atom coordinated by four nitrogen atoms of the corrin ring, as shown in Scheme 1. Under non-reducing conditions, the cobalt atom commonly exists in the +3 oxidation state (cob(III)alamin), axially coordinating two ligands (methyl or cyanide group in the “upper” and 5,6-dimethylbenzimidazole (DMB) in the “lower”).\textsuperscript{6,7} In abiotic systems, cob(III)alamin can be reduced to 4-coordinated cob(I)alamin without axial ligands in the presence of strong reducing agent in aqueous media,\textsuperscript{8,9} while this model system mimicking microbial dehalogenation has been used in abiotic remediation strategies for treatment of contaminated field sites.\textsuperscript{10} Compared with the rigorous selectivity of enzymatic systems, in vitro studies have shown that cob(I)alamin can catalyze nonspecific reductive dehalogenation of many halogenated compounds, such as chlorinated methanes, ethanes,
higher alkanes, ethylenes, arenes, etc.\textsuperscript{11-12} Thus \textbf{Accordingly}, the abiotically reductive dehalogenation by cobalamin has substantial potential for use in remediation approaches,\textsuperscript{10, 13-14} while some other transition metal complexes (e.g. iron porphyrin) as well as nano-scale zero-valent metal systems (e.g. iron, zinc, \textit{et al.}) have also been reported to be \textit{potentially useful} reductive dehalogenation catalysts.\textsuperscript{15-17} Practically, degradation of halogenated compounds by reductive dehalogenation reactions may occur via numerous routes. In order to assess whether the dehalogenation brings about significant detoxification, the fundamental knowledge of the reductive dehalogenation mechanisms involving cobalamin is essential. However, cob(I)alamin contains the unusual combination of properties that it is \textit{of being} both a strong reductant,\textsuperscript{18} and one of the most powerful nucleophiles that is $\sim 10^4$ times more nucleophilic than the Cl anion in $\text{SN}_2$ reactions,\textsuperscript{19} which makes the reaction modes more diverse and complex.

**Scheme 1.** Structure of the Cobalamin

\textbf{Commented [KPK2]}: I don’t think I mention this number specifically, perhaps another reference?

\textbf{Commented [KPK3]}: I think this should be a figure not a scheme – a scheme is e.g. a reaction process.
Among various halogenated compounds, the mechanistic study of cobalamin–mediated reductive dehalogenation of chloroethylenes has attracted particular attention. Cob(I)alamin has been reported to participate in the sequential dehalogenation of perchloroethylene (PCE), trichloroethylene (TCE), cis-1,2-dichloroethylene (cis-DCE), trans-1,2-dichloroethylene (trans-DCE), and vinyl chloride (VC), resulting in the production of nontoxic ethylene.9, 20-25 As shown in Scheme 2, the initial step for cob(I)alamin-catalyzed chloroethylenes can be summarized as an outer-sphere (reaction occurring between chemical species with remaining in a non-connected state) or an inner-sphere (with a chemical bond forming between chemical species during the reaction) process, which can be categorized in more detail more specifically as single electron-transfer [outer-sphere (path a)], nucleophilic substitution [inner-sphere (path b)], and nucleophilic addition [inner-sphere (path c)] mechanisms. Most previous work focused on the reductive dehalogenation mechanism of highly-chlorinated substances, PCE and TCE.9, 21, 23-25 On the basis of kinetic experiments, the pH-independent rate constants were observed, which may rules out inner-sphere nucleophilic addition route—proceeding with simultaneous protonation.21-22 Meanwhile, cob(I)alamin-mediated dehalogenation of PCE and TCE with increasing amounts of d7-isopropanol, a D• donor, resulted in as-at most 10% of the PCE-derived deuterated products and 30% of the TCE-derived deuterated products, in agreement with outer-sphere one-electron transfer.21 However, this outer-sphere mechanism is not in accord with stereochemical results that the dehalogenation of TCE by cob(I)alamin produces greater amount of cis-DCE compared to trans-DCE (> 15:1), markedly different from the ratio obtained with identified electron-transfer reagents (< 5:1).23 Support for the nucleophilic substitution mechanism originated from experimental observation that the molecular mass consistent with dichlorovinylcobalamin had been observed in mass spectra during the TCE dehalogenation reaction.25 If this nucleophilic
substitution mechanism works for PCE with cob(I)alamin as well, the trichlorovinylcobalamin from the PCE dehalogenation reaction should be detected, but this has not been the case.\textsuperscript{11}

The experimental work performed with isopropyl alcohol-d\textsubscript{7} at different concentrations has showed that, in contrast to PCE and TCE, there were not marked\textsuperscript{were only few} deuterated products captured for the cobalamin-catalyzed reductive dehalogenation of less-chlorinated substances, \textit{cis}-DCE, \textit{trans}-DCE, and VC. \textsuperscript{4 This indicates that} there is not significant quantities\textsuperscript{of free radicals produced \textit{during} the dehalogenation process. Meanwhile, the kinetic experiments have shown that cobalamin reductively dehalogenated\textsuperscript{cis-DCE, trans-DCE, and VC in pH-dependent reactions}.\textsuperscript{22}

\textbf{Scheme 2. Alternative Reaction Mechanisms for Reductive Dehalogenation of Chloroethylene Catalyzed by Cobalamin}\textsuperscript{a}

\textsuperscript{a} taking PCE as an example

Isotope fractionation \textit{during} reductive dehalogenation of chloroethylenes with cobalamin has been investigated using compound specific isotope analysis (CSIA).\textsuperscript{26-31} CSIA is able to...
offers new insight into the organic pollutant degradation mechanism, especially when the competing reaction pathways are unknown. In the process of cobalamin-mediated reductive dehalogenation of chloroethylenes, most previous work focused on stable carbon isotopes, and stable chloride isotopes were not commonly studied. For example, the reported carbon bulk isotope fractionation factors ($\varepsilon_{\text{bulk,C}}$) are $-15.8\%$ for PCE and $-16.1\%$ for TCE; however, in contrast to cobalamin-catalyzed PCE and TCE, the reported values for cis-DCE ($-25.5\%$) and VC ($-31.1\%$) are much larger, which may indicate an entirely different reductive dehalogenation mechanism. Until now, no systematic computational investigation of isotope fractionation within the different operative pathways for reductive dehalogenation of chloroethylenes in the presence of cobalamin has been undertaken although such investigation should shed light onto this ongoing mechanistic debate.

Computational analysis of the catalytic mechanism can provide insight into the electronic structure features governing reaction mechanisms, which has already been performed to give such studies have provided insight into the viability of various intermediates and pathways in the reductive dehalogenation of chloroethylenes with cobalamin as well as synthetic cobaloximes. More specifically, the computed electrochemical properties of the reduced chloroethylenes and chlorinated-cobalamins have been useful for interpreting some experimental observations, such as indicating that the formed chlorinated vinyl radicals during reductive dehalogenation may be reduced to anionic forms competing with their rebound to cob(I)alamin to produce vinylcobalamins. It is noteworthy that Notably, most work has used the cobalamin structure simplified through cutting off the corrin model without side chains and replacing the axial DMB base with imidazole instead of DMB for studying the mechanism of reductive dehalogenation, yet these substituents can affect cobalamin electron structure substantially, so

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while the influence of omitting the substituents and substitution of using the simpler axial base needs to be addressed. Until now, the precise reductive dehalogenation mechanism has not been established in details that would warrant explanation of all experimental observations collected so far.

In this work we use density functional theory (DFT) on realistic full cobalamin structures to address the following unsolved mechanistic questions: (i) why pH-independent rate constants have only been observed for the reactions of PCE and TCE with cobalamin, but not for cobalamin-catalyzed reactions of cis-DCE, trans-DCE, and VC; (ii) why there is conflicting evidence for the dehalogenation processes of cobalamin-mediated PCE and TCE favoring either the outer-sphere or the inner-sphere pathway, respectively; (iii) why only dichlorovinylcobalamin has been detected upon reaction of TCE with cobalamin, but not trichlorovinylcobalamin in the reaction of cobalamin-mediated PCE; (iv) whether the comparison between calculated kinetic isotope effects (KIEs) and experimental apparent kinetic isotope effects (AKIEs) can be used to identify the competing dehalogenation pathways.

Computational Methodology

The System for Cobalamin-Mediated Chloroethylenes Computational Details. All calculations in this work were performed with the Gaussian 09 Revision D.01 program package.

The complete 4-coordinated cob(I)alamin species (with the nucleotide loop is clipped off), was used as initial structure basis for our computational work. The geometry optimizations and frequency analyses have been carried out in the gas phase using the Perdew-Burke-Ernzerhof (PBE) functional,\textsuperscript{49-50} combined with Ahlrich’s TZV basis set\textsuperscript{51} for Co and 6-31G** basis set\textsuperscript{52} for C, N, H, O and Cl (denoted as BSI). The PBE functional has been previously shown...
to accurately reproduce the experimental frequencies of the corrin-based normal modes of vibration, and yields geometries in accordance with experimental cob(I)alamin structural parameters from XAS studies and accurate Co-C bond dissociation energies. Subsequent frequency calculations were run to confirm that all ground states had only real frequencies, whereas the transition states had one imaginary frequency. The computed vibrational frequencies were further used to quantify the zero-point energy correction (ZPE), and enabled us to convert the electronic energy to the Gibbs free energy at 298.15 K and 101.325 kPa. The intrinsic reaction coordinate (IRC) calculations were performed to verify that the transition states connected the reactants and products. Based on the PBE-optimized structures in the gas phase, the water solvation effects were calculated by COSMO continuum-solvation model (CPCM, dielectric constant = 78.3) at the PBE/BSI level of theory. The dispersion interactions were considered through single-point calculations at the PBE-D3/BSI level of theory due to the standard DFT method lacking of such interactions. Unless otherwise specified, all relative free energies for cobalamin-mediated chloroethylenes reactions reported were based on PBE/BSI data including solvation and D3 dispersion corrections \( \Delta G + E_{\text{solv}} + E_{\text{disp}} \). We also performed PBE-D3 geometry optimizations with CPCM in water solution on five structures of cob(I)alamin, cob(II)alamin, base-off trichlorovinylcob(III)alamin, base-on trichlorovinylcob(III)alamin and base-on trichlorovinylcob(II)alamin to verify the reliability of the above method approach, the results of which show that the effects of dispersion and solution are negligible on the optimized geometries (the geometrical comparison between PBE/BSI optimized structures in the gas phase and PBE-D3-CPCM/BSI optimized structures is shown in Figure S9 in the Supporting Information).
Cobalamin chemistry is generally low-spin, as implied by the ground state of cob(I)alamin and cob(III)alamin with singlet state and cob(II)alamin with doublet state. As further proof, the calculations in this work at the PBE/BSI level of theory-free energy including solvation and D3 dispersion corrections show for cob(I)alamin and cob(III)alamin (base-off trichlorovinylcob(III)alamin) on reveal triplet states are at 21.3 and 18.4 kcal/mol respectively higher than the singlet state species, while the cob(II)alamin on high-spin quartet state is 29.1 kcal/mol higher than the low-spin doublet state species; thus, in this work these species were all optimized in the low-spin state.

Particular attention has been focused on the electronic structure of cob(I)alamin. Previous computational studies have investigated the electronic structure of four-coordinated cob(I)alamin without the axial DMB base and five-coordinated cob(I)alamin with the axial DMB base using DFT and CASSCF calculations. The TD-DFT calculations on truncated four-coordinated cob(I)alamin have suggested that ground state cob(I)alamin is purely a closed-shell singlet d^8 species. Subsequent CASSCF calculations on the truncated cob(I)alamin have shown that the dominant contribution to the ground state wave function is the closed-shell singlet d^8 Co(I) configuration for four-coordinated cob(I)alamin and for five-coordinated cob(I)alamin with a weakly coordinated axial base, while cob(I)alamin is mainly dominantly the open-shell singlet/triplet d^7 Co(II)-corrin (π*) diradical configuration with a strongly coordinated axial base. The reaction mechanisms of methyl transfer between cob(I)alamin and CH3H4folate catalyzed by methionine synthase were revealed have been studied computationally, with the four-coordinated base-off cob(I)alamin conformation for studying the Sn2 pathway and the five-coordinated base-on cob(I)alamin conformation for studying the electron-transfer pathway, giving similar barriers for these two pathways. However, while...
cob(I)alamin only existing in the enzyme can coordinate to the axial DMB base due to the H-bonding between the base and nearby amino acid residues. The X-ray absorption spectroscopic experiments have shown that cob(I)alamin in solution is not axially coordinated to DMB base at all, which is proved again in this work that PBE calculations show the complete five-coordinated cob(I)alamin on open-shell singlet/triplet state is about 22 kcal/mol higher in free energy at PBE/BSI level of theory including solvation and D3 dispersion corrections than the complete four-coordinated cob(I)alamin on close-shell singlet state plus DMB base in solution. Supporting the strict low-spin closed-shell configurations as found in our study. Furthermore, in this work, the closed-shell singlet stability of the complete four-coordinated cob(I)alamin was proved by mixing the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) from PBE orbitals using PBE functional, and the by CASSCF single-point calculation on the complete (not truncated) four-coordinated cob(I)alamin. Many studies have also shown the experimentally calibrated PBE and BP86 methods are accurate for ground-state thermochemistry of cobalmins, partly because the required active spaces from CASSCF e.g. CASPT2 methods are out of range to be computationally tractable, and partly because of the basis set requirements. To summarize what has been mentioned above, this work focuses on the four-coordinated cob(I)alamin on the closed-shell singlet state using PBE method to study the reductive dehalogenation mechanisms.

**Electrochemical Properties of Chlorinated Cobalamin.** The aqueous-phase standard reduction potentials ($E^0$) referenced with respect to the standard calomel electrode (SCE) ($E^0$ vs. SCE, unit: V) of all chlorinated vinyl- and ethyl-cobalamins as well as the cob(II)alamin/cob(I)alamin couple were converted from the aqueous-phase adiabatic electron
The absolute reduction potential of SCE for cobalamin was applied as recommended before, as shown in eq 1:

\[ E^0_{\text{vs. SCE}} (V) = \text{AEA} - 4.52 \]  

1

The AEA values were in the form of free energy changes, with using the PBE single-point calculations obtained from the PBE/BSI-optimized geometries using the larger 6-311+G(2d,2p) basis set for main group atoms and TZV for cobalt (denoted as BSII), including water solvation energy and D3 dispersion corrections (BSII level of theory), and free energy corrections (BSI level of theory). The vertical electron affinities (VEA) for the base-off and base-on less-chlorinated ethylcobalamins were obtained from PBE/BSII//BSI single-point calculations with water solvation and D3 dispersion corrections.

Electrochemical Properties and Electrophilic Reactivity of Chloroethylenes. All calculations for the electrochemical properties of chloroethylenes were performed using the PBE/aug-cc-pVTZ level of theory (BSIII) with CPCM solvation model of aqueous solution (dielectric constant = 78.3). VEA for all chloroethylenes are electronic energy differences in aqueous-phase water, while adiabatic electron affinities for all vinyl radicals are aqueous-phase free energy change in waters. The adiabatic electron affinities of vinyl radicals were translated into aqueous-phase E^0 vs. SCE. The Electrophilic index (\( \omega \)), developed from the concept of the hard and soft acids and bases (HSAB), were calculated to characterize the electrophilic reactivity of chloroethylenes. In order to calculate the electrophilic index (\( \omega \)), firstly the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) energies were calculated at the BSIII level of theory in the gas phase, thusFrom these, it is possible to
quantify the three basic HSAB parameters as follows: hardness ($\eta$) as $(E_{\text{LUMO}} - E_{\text{HOMO}})/2$, softness ($\sigma$), defined as the inverse of hardness ($1/\eta$), and the chemical potential ($\mu$), as $(E_{\text{LUMO}} + E_{\text{HOMO}})/2$.

Then, the electrophilic index ($\omega$) was calculated as $\mu^2/\eta$.

**Kinetic Data.** The reaction rate constant of for reaction of the chloroethylenes with cob(I)alamin, k, and the corresponding free energy of activation, $\Delta G^\neq$, can be converted to each other according to the Eyring equation (eq 2):

$$k = \frac{k_B T}{h} \cdot \frac{1}{c^0} \exp \left( -\frac{\Delta G^\neq}{RT} \right)$$

where $k_B$ is the Boltzmann constant, $h$ is the Planck constant, $R$ is the gas constant, $T$ the temperature in Kelvins, and $c^0$ is the concentration defining the standard state (typically 1 mol/L). This equation was used to estimate relative rate constants from activation barriers.

**Isotope Effects.** The Hessians obtained from the above-mentioned frequency calculations after geometry optimizations were used to calculate kinetic isotope effects (KIEs) with the ISOEFF package. KIEs were obtained according to the Bigeleisen equation at 298 K for the transition from two separate reactants to the corresponding transition state. The apparent kinetic isotope effects (AKIE) value from experiments can be approximated from using the bulk isotope fractionation factors ($\varepsilon_{\text{bulk}}$) by eq 3:

$$\text{AKIE} \approx \frac{1}{1 + n/x \cdot z \cdot \varepsilon_{\text{bulk}}}$$

where $n$ is the number of atoms of the considered element, $x$ is the number of atoms of the considered element at the reactive position, and $z$ is number of atoms of the considered element in intramolecular isotopic competition. It should be noted that in this form the secondary isotope effects are neglected, an assumption that should be plausible for chlorine KIEs.
All calculations in this work were performed with the Gaussian 09 Revision D.01 program package.22

Results and Discussion.

Calibration vs. Experimental Data.

Based on DFT optimization results with the PBE functional, our DFT computations show that two-electron reduction of the complete model of cob(III)alamin to cob(I)alamin leads to both the axial methyl and DMB groups leaving off, providing the 4-coordinated cob(I)alamin species [as shown in Figure 1], as expected. The corrin macrocycle in the complete cob(I)alamin model is almost planar with averaged Co-N bond length of 1.86 Å. This is consistent with the average Co-N bond lengths of 1.86 and 1.88 Å reported in two recent XAS studies.53-54 In addition, the calculated standard reduction potential (E₀) value for the cob(II)alamin/cob(I)alamin couple is −0.78 V vs. SCE, near to the corresponding experimental data of −0.85 V vs. SCE, further validating the reliability of the theory level used.

Figure 1. Chemical Structure along with Bond Lengths of Planar Co-N Bonds (Å) for Cob(I)alamin.
Recently, the dispersion-driven O-H⋯Pt interaction between trans-[PtCl₂(NH₃)(N-\[glycine\]) and water molecular has been revealed experimentally at very low temperature, which supports indicating that the d⁸ metal ions in complexes can act as H-bonding acceptors. Considering the fact that the dominant contribution to the ground state is the closed-shell singlet d⁸ Co(I) for cob(I)alamin, a scientific question the possibility arises that whether the Co(I)⋯H interaction between cob(I)alamin and a water molecular can also be formed and consequently influence the cob(II)alamin/cob(I)alamin reduction. Relevant computational computations have been done, which confirms the possibility of forming the a Co(I)⋯H linkage between cob(I)alamin and water molecular from thermodynamics, and suggests that this unusual Co(I)⋯H interaction may have significant catalytic relevance during the reactivation cycle of the methionine synthase enzyme.⁷¹ Therefore, the Co(I)⋯H as well as Co(I)⋯Cl interactions between cob(I)alamin and chloroethylenes have been explored in this work. As shown in Table 1, all Co(I)⋯H and Co(I)⋯Cl bond formation processes between cob(I)alamin and different chloroethylenes are endothermic in water solution based on the PBE/BSI free energies including solvation and D3 dispersion corrections, which seem to be nonspontaneous from thermodynamics. This is in accord with previous computations suggesting the earlier computational study that the Co(I)⋯H bond formation between cob(I)alamin and a water molecular in water solution was not favorable in water solution where water-water interactions are favored, and in line with previous experiments indicating all work that there was no such observed H-bonding in water solution. Therefore, this work uses the two separate reactants (i.e., cob(I)alamin and chloroethylene) as starting point to study the reductive dehalogenation mechanisms in the following sections. However, since the earlier computational computations work showed that the nonpolar solvent such as chloroform (a solvent

Commented [KPK15]: Chloroform is polar just not as much as water
mimicking a dielectric constant similar to that of a typical protein environment) makes the Co(I)···H bond formation process favorable/feasible, this unusual Co(I)···H or even Co(I)···Cl bonding needs to be considered for here in the context of reductive dehalogenation catalyzed by cob(I)alamin in an enzyme.

Table 1. Computed Free Energies (kcal/mol) at the PBE/BSI level of theory with solvation and D3 dispersion corrections for Co(I)···H and Co(I)···Cl Bond Formation between Cob(I)alamin and Chloroethylenes in Water Solution

<table>
<thead>
<tr>
<th>Co(I)···H</th>
<th>Co(I)···Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td>AG</td>
<td>AG</td>
</tr>
<tr>
<td>4.9</td>
<td>9.7</td>
</tr>
<tr>
<td>4.5</td>
<td>8.0</td>
</tr>
<tr>
<td>4.0</td>
<td>9.5</td>
</tr>
<tr>
<td>7.4</td>
<td>9.1</td>
</tr>
<tr>
<td>4.8</td>
<td>8.9</td>
</tr>
<tr>
<td>3.3</td>
<td>9.0</td>
</tr>
<tr>
<td>2.9</td>
<td>7.3</td>
</tr>
</tbody>
</table>

Outer-Sphere Electron Transfer Mechanism.

We first focus on the outer-sphere electron transfer pathway of cob(I)alamin catalyzing chloroethylenes according to eq.4:

\[
\text{Co}^\text{II} + \text{DMB} + \text{Cl} \longrightarrow \text{Cl} + \text{Cl} + \text{Cl} \rightarrow \text{Co}^\text{III} \text{DMB} \tag{4}
\]
DMB: 5,6-dimethylbenzimidazole

The free energy barriers of the outer-sphere electron transfer processes ($\Delta G^{\neq}_{ET}$) can be estimated from the Marcus theory\textsuperscript{75-78} (calculation details shown in the Supporting Information). The obtained $\Delta G^{\neq}_{ET}$, the free energy of reaction ($\Delta G_{ET}$) as well as the vertical electron affinities (VEA) values for all chloroethylenes are shown in Table 2. Both $\Delta G^{\neq}_{ET}$ and $\Delta G_{ET}$ values increase in the sequence PCE < TCE < trans-DCE < cis-DCE < VC, with increasing number of chlorine atoms and decreasing of the vertical electron affinities (VEA) values. Then a linear free energy relationship (LFER) between $\Delta G_{ET}$ and VEA values for the one-electron-transfer reaction was built.

As a result, the VEA values are closely correlated to the free energy barriers with an $r^2$ of 0.940 ($\Delta G^{\neq}_{ET} = -67.02\text{VEA} + 104.11$) (in kcal/mol). Therefore, it is possible to provide a computationally less demanding tool for preliminary evaluation of the free energy barriers of the electron transfer process for cobalamin-mediated reductive dehalogenation of halogenated compounds within one class.

**Table 2.** The Free Energies (kcal/mol) of the Electron-Transfer Reactions for Cobalamin-Mediated Reductive Dehalogenation of Chloroethylenes, along with the Vertical Electron Affinities (VEA, eV) of Chloroethylenes

<table>
<thead>
<tr>
<th>PCE</th>
<th>TCE</th>
<th>cis-DCE</th>
<th>trans-DCE</th>
<th>VC$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEA</td>
<td>1.36</td>
<td>1.24</td>
<td>1.02</td>
<td>1.13</td>
</tr>
<tr>
<td>$\Delta G^{\neq}_{ET}$</td>
<td>15.9</td>
<td>17.6</td>
<td>34.7</td>
<td>27.3</td>
</tr>
<tr>
<td>$\Delta G_{ET}$</td>
<td>15.6</td>
<td>17.2</td>
<td>31.0</td>
<td>25.7</td>
</tr>
</tbody>
</table>

$^a$VC: vinyl chloride

Note that the outer-sphere electron transfer reactions for all chloroethylenes by cob(I)alamin are highly endergonic—however, the calculated standard reduction potentials ($E^0$) of
vinyl radicals listed in Table S3 in the Supporting Information) show that all chlorinated vinyl radicals with E° values between −0.40 V to 0.16 V, i.e., they can be reduced at standard conditions by cob(I)alamin, which could provide additional driving force for the reaction to proceed. The free energy barrier values for PCE and TCE are considerably lower (< 20 kcal/mol), implicating indicating that the outer-sphere electron transfer reactions for cob(I)alamin catalyzing PCE and TCE could take place at normal temperatures, but such reactions are hampered by the high barrier for cob(I)alamin catalyzing trans-DCE, cis-DCE and VC. These results are consistent with experimental findings; that no significant amount of free vinyl radicals were produced in the reactions of cob(I)alamin reducing trans-DCE, cis-DCE and VC, while significant chlorinated vinyl radicals formed during the reactions of PCE and TCE catalyzed by cob(I)alamin. In the following sections, the competition between the outer-sphere and inner-sphere reduction of chloroethylenes with cob(I)alamin will be addressed.

**Inner-Sphere Nucleophilic Substitution Mechanism.**

Figure 2 shows the free energy profile for the inner-sphere nucleophilic substitution of PCE with cob(I)alamin, together with geometric details of the relevant molecular species. From separated reactants, cob(I)alamin + PCE, the nucleophilic substitution reaction takes place via the concerted transition state TSNS, associated with a barrier of 14.2 kcal/mol, leading synchronously to base-off trichlorovinylcob(III)alamin under upon loss of chloride anion \( \text{Cl}^- \) (the Mulliken charge of dissociated \( \text{Cl}^- \) changes from 0.11 in the reactants to -0.65 in the products), slightly exothermic of ~0.2 kcal/mol relative to reactants. The experimental second-order rate constant of cob(I)alamin-mediated PCE varied slightly with pH \( [\text{pH}] = 9 \), from \( 125 \pm 7 \) to \( 179 \pm 10 \, \text{M}^{-1}\text{s}^{-1} \). Thus bringing the kinetic information into using the Eyring equation (eq 2), yields a free energy...
barrier from 14.3 to 14.6 kcal/mol, very close to the above calculated nucleophilic substitution barrier of PCE with cob(I)alamin. The qualitative comparison of the free energy barriers of nucleophilic substitution (ΔG\text{NS} = 14.2 kcal/mol) and electron transfer (ΔG\text{ET} = 15.9 kcal/mol) estimates the pathway ratio of nucleophilic substitution to electron transfer of ~17:1, in accord with the experimental phenomenon that maximally ten percent free radicals are formed during dehalogenation of PCE with cob(I)alamin, resulting in the ratio for inner-sphere pathway to out-sphere pathway of more than 10:1.21 No minima along the reaction path corresponding to initial cob(I)alamin • PCE adduct could be located, and the IRC calculations verified the reaction path leading down from the transition state to separate cob(I)alamin and PCE as reactants (reverse direction) and base-off trichlorovinylcob(III)alamin as product (forward direction), as shown in Figure S3 in the Supporting Information. The characteristic S_N2 transition-state geometry, with bond making occurring simultaneously with bond breaking, is easily recognized. This S_N2 transition-state geometry is rather unsymmetrical with the forming C-Co bond (2.05 Å) much shorter than the breaking C-Cl bond (2.40 Å).
**Figure 2.** Free Energy Profile (kcal/mol) of Cob(I)alamin-mediated Reductive Dehalogenation of PCE, along with the Optimized Geometries (Å) of the Key Species and the Imaginary Frequency in Transition State in wave numbers. Relative Energies were given in the Form of $\Delta G + E_{solv} + E_{disp}$.

Electrochemical experiments and DFT calculations have demonstrated that the one-electron-reduced alkyl-cobalt complexes exhibit significant lowering of the Co-C bond dissociation energy in comparison to their neutral precursors. Therefore, once the trichlorovinylcobalamin is formed, it is essential to investigate the subsequent reductive cleavage processes of Co-C bond for both the base-off and base-on trichlorovinylcobalamins, to give the dehalogenation product of PCE, i.e., TCE. The calculated $E^0$ for base-off and base-on trichlorovinylcobalamins are $-0.63$ V vs. SCE and $-0.58$ V vs. SCE, respectively. In combination with the calculated $E^0$ of $-0.78$ V vs. SCE and experimental $E^0$ of $-0.85$ V vs. SCE for the cob(II)alamin/cob(I)alamin couple, it demonstrates that both the base-off and base-on trichlorovinylcobalamins could be readily reduced under the reductive reaction conditions.

**Figure 3** shows the optimized structures for both the one-electron-reduced base-off and base-on trichlorovinylcobalamin (trichlorovinylcob(II)alamin). It is remarkable that the DMB base dissociates far away from the cobalt center with Co-N length of 5.0 Å for the base-on trichlorovinylcob(II)alamin, suggesting the base-on trichlorovinylcob(II)alamin probably has both the “base-on” and “base-off” properties. The complete base-on trichlorovinylcob(II)alamin with the loose axial DMB base is quite different from the previously reported simplified base-on trichlorovinylcob(II)alamin with the tight axial imidazole base, partly because DMB is a weaker donor ligand than imidazole and partly due to steric repulsion. Subsequently, cleavage of the Co-
C bond of trichlorovinylcob(II)alamin may occur homolytically to form a vinyl anion and cob(II)alamin, or heterolytically to form a vinyl radical and cob(I)alamin, as shown in Scheme 3. The calculated free energies for homolysis and heterolysis of the base-off form are 29.6 and 11.0 kcal/mol, respectively (18.1 and 13.9 kcal/mol without dispersion correction), while the corresponding values for the base-on form are 21.8 and 3.2 kcal/mol (−3.5 and −7.7 kcal/mol without dispersion correction). Thus, the heterolytic cleavage of the Co-C bond of the base-on trichlorovinylcob(II)alamin is the most favorable pathway, which is similar to the previous theoretical examination of the Co-C cleavage of reduced cis-dichlorovinylcobaloxime.  

Figure 3. The Optimized Structures of Base-off Trichlorovinylcob(II)alamin (a) and Base-on Trichlorovinylcob(II)alamin (b).

Scheme 3. Homolysis and Heterolysis of the Base-on and Base-off Forms of Trichlorovinylcob(II)alamin
We then investigated the nucleophilic substitution mechanism of TCE, cis-DCE, trans-DCE and VC. Table 3 summarizes the relative energies of inner-sphere nucleophilic substitution processes for cobalamin-mediated all chloroethylenes as well as the electrophilic index ($\omega$) for all chloroethylenes. The optimized geometries of the $S_n$2 transition-states are shown in Figure S1 in the Supporting Information. All the reaction paths were verified by IRC calculations that the transition state leading to separate cob(I)alamin and chloroethylene as reactants in the reverse direction and base-off vinylcob(I)alamin as product in the forward direction, as shown in Figure S4−S8 in the Supporting Information. Similarly, for the nucleophilic substitution mechanism of TCE with cob(I)alamin, no initial cob(I)alamin•chloroethylene adduct could be located in the reaction path. It is obvious that as seen, the free energy barriers of nucleophilic substitution are generally increasing with decreasing of number of chlorine atoms in chloroethylenes, with an exception of dehalogenation of TCE to trans-DCE with larger barriers (minor pathway for dehalogenation of TCE). Moreover, quantitatively, increasing the electrophilic reactivity of chloroethylenes as quantified through the electrophilic index ($\omega$) decreases the nucleophilic substitution barrier, thus yielding a good correlation with an $r^2$ value of 0.938 ($\Delta G^{nS} = -0.195\omega + 6.95$). This result indicates the suitability of $\omega$ to screen the reactivity of cobalamin-mediated reductive dehalogenation of halogenated compounds in vitro the inner-sphere nucleophilic substitution pathway.

Table 3. The Relative Free Energies (kcal/mol) for Cobalamin-mediated Reductive Dehalogenation of Chloroethylenes during the Inner-Sphere Nucleophilic Substitution Processes along with the Electrophilic Index ($\omega$) of Chloroethylenes

<table>
<thead>
<tr>
<th></th>
<th>PCE</th>
<th>TCE*</th>
<th>TCE*</th>
<th>cis-DCE</th>
<th>trans-DCE</th>
<th>VC</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TCE</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>TCE*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\omega$</td>
<td>4.12</td>
<td>3.83</td>
<td>3.83</td>
<td>3.34</td>
<td>3.59</td>
<td>3.12</td>
</tr>
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<td>---</td>
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<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>$\text{TSS}$</td>
<td>14.2</td>
<td>16.9</td>
<td>18.6</td>
<td>18.2</td>
<td>17.3</td>
<td>19.5</td>
</tr>
<tr>
<td>$P_{\text{SS}}$</td>
<td>-0.2</td>
<td>-7.9</td>
<td>-1.8</td>
<td>-5.1</td>
<td>-6.0</td>
<td>-12.5</td>
</tr>
</tbody>
</table>

*dehalogenation of TCE to produce cis-DCE; §dehalogenation of TCE to produce trans-DCE

It is apparent that conversion of TCE may produce cis-DCE and trans-DCE, respectively.

From the relative energies in Table 3, cis-DCE is the main product under both kinetic and thermodynamic control. The computational kinetic data from the Eyring equation (eq 2) predicts a preponderance of produced cis-DCE over trans-DCE by a factor of 18 to 1, which is in excellent agreement with the products distribution of cobalamin-mediated dehalogenation of TCE found experimentally (cis-DCE : trans-DCE ratios > 15 : 1).\(^{20,21}\) The energy barrier for conversion of TCE into cis-DCE gives the rate constant of 2.5 M\(^{-1}\)s\(^{-1}\), almost the same as the experimental data from 2.4 ± 0.2 M\(^{-1}\)s\(^{-1}\) to 3 ± 0.1 M\(^{-1}\)s\(^{-1}\).\(^{21}\) Then, combining with the above obtained free energy barrier of the electron-transfer process ($\Delta G^{\text{ET}}_{\text{F}} = 17.6$ kcal/mol) for TCE with cob(I)alamin, the ratio of nucleophilic substitution pathway to electron transfer pathway is predicted to be 3.5 : 1 through the Eyring equation (eq 2), consistent with the experimentally determined ratio for inner-sphere pathway to out-sphere pathway of > 2.3 : 1.\(^{21}\)

Moreover, the reaction barriers of cob(I)alamin-mediated dehalogenation of cis-DCE, trans-DCE and VC are within 20 kcal/mol. Thus, accordingly, the nucleophilic substitution reactions for these less-chlorinated ethylenes by cobalamin could happen in theory in principle occur. However, the previous experimental work had shown that the increase of pH by one unit lead to a decrease of the reaction rate by roughly a factor of ten, suggesting that a proton was involved in the rate-determining step,\(^{22}\) which contradicts the inner-sphere nucleophilic substitution pathway, to be discussed below.
The results in former section above suggest that the trichlorovinylcobalamin could be rapidly reduced under reductive conditions. Therefore, it is necessary to investigate the redox potentials of other less-chlorinated vinylcobalamins to know whether they can be reduced under similar conditions. Due to the preponderance production of more cis-DCE over trans-DCE for when TCE reacts with cobalamin, the following work only focused on cis-dichlorovinylcobalamin (dichlorovinylcobalamin). As shown in Table 4, the calculated $E^0$ values are becoming more negative with decreasing chlorine atoms in the chlorinated vinylcobalamins, and the $E^0$ values for all base-on dichlorovinylcobalamin, cis- and trans-chlorovinylcobalamin, and vinylcobalamin are more negative than their base-off forms. Especially in particular, the $E^0$ value for the base-off dichlorovinylcobalamin (~0.86 V vs. SCE) is substantially higher than for the corresponding base-on form (~1.23 V vs. SCE), while the $E^0$ values for the base-off chlorovinylcobalamins and vinylcobalamins are only a bit more positive than for their base-on forms. It is obvious that among these less-chlorinated vinylcobalamins, only the base-off dichlorovinylcobalamin would appear as a candidate for promotion of reduction by cob(I)alamin, although the $E^0$ value for the base-off dichlorovinylcobalamin (~0.86 V vs. SCE) is a bit more negative than the $E^0$ value for the cob(II)alamin/cob(I)alamin couple (experimental value: ~0.85 V vs. SCE; calculated value: ~0.78 V vs. SCE). All other base-off and base-on chlorinated vinylcobalamins are not feasible intermediates due to their much more negative $E^0$ value than the cob(II)alamin/cob(I)alamin couple.

Table 4. Computed Aqueous-Phase Standard Reduction Potentials ($E^0$) (V vs. SCE) for the Base-off and Base-on Vinylcobalamins

<table>
<thead>
<tr>
<th></th>
<th>trichlorovinyl cobalamin</th>
<th>dichlorovinyl cobalamin</th>
<th>cis-chlorovinyl cobalamin</th>
<th>trans-chlorovinyl cobalamin</th>
<th>vinyl cobalamin</th>
</tr>
</thead>
</table>

Commented [KPK25]: This is also very accurate; absolute potentials have errors that mimic the errors in functionals for IPs, i.e. 5 kcal/mol. Were they calibrated? Perhaps discuss why they agree so well.
After formation of the one-electron-reduced base-off form of dichlorovinylcobalamin (dichlorovinylcob(II)alamin), the corresponding base-on form with re-coordination of the DMB base to the Co center may be formed. As shown in Figure 4, different from the base-on in contrast to trichlorovinylcob(II)alamin, the DMB base coordinates strongly with the cobalt center for the to produce base-on cis-dichlorovinylcob(II)alamin. The geometry difference between the base-on trichlorovinylcob(II)alamin and dichlorovinylcob(II)alamin may eome—arise from the much stronger inductive effect of the trichlorovinyl-fragment (Mulliken charge: −0.71) compared to the dichlorovinyl-fragment (Mulliken charge: −0.44) (more detailed electronic structure analysis is given in the following partbelow). We then calculated the free energy changes of the Co-C bond cleavage for both the base-off and base-on dichlorovinylcob(II)alamin. As for trichlorovinylcob(II)alamin, heterolysis in the base-on forms is the thermodynamically preferred mode of Co-C bond cleavage with a free energy of 7.7 kcal/mol (−7.2 kcal/mol without dispersion correction) (for the detailed free energy comparisons see Table S26 in the Supporting Information).
Figure 4. The Optimized Structures of Base-off Dichlorovinylcob(II)alamin (a) and Base-on Dichlorovinylcob(II)alamin (b).

It is noteworthy that detection of trichlorovinylcobalamin has never been successful, but efforts to detect dichlorovinylcobalamin have been feasible.\textsuperscript{25} This has been a long-term unsolved mechanistic topic in cobalamin chemistry.\textsuperscript{11} As mentioned above, the base-on trichlorovinylcob(II)alamin with quite long Co-N length shown in Figure 3 is close to its base-off form, making the reduction potential of the base-on trichlorovinylcobalamin (−0.58 V vs. SCE) as negative as the base-off form (−0.63 V vs. SCE), \textit{i.e.} the base-on trichlorovinylcobalamin is easily reduced under reductive conditions. By contrast, the tight Co-N bond with bond length of 2.2 Å in the base-on dichlorovinylcob(II)alamin shown in Figure 4 causes the reduction potential of the base-on dichlorovinylcobalamin (−1.23 V vs. SCE) to be much more negative than its base-off form (−0.86 V vs. SCE), \textit{resulting in implying a relatively longer lifetime for the base-on dichlorovinylcobalamin. Thus, it is possible to observe the mass consistent with the dichlorovinylcobalamin in mass spectra of the TCE dehalogenation reaction. On the other hand, although the DMB base dissociates far away from the cobalt center for the base-on trichlorovinylcob(II)alamin, the \textit{strong}-destabilization \textit{effect} by the nitrogen lone pair electrons from the axial of DMB ligand—results in a weaker Co-C bond for the base-on trichlorovinylcob(II)alamin than its base-off form, and its Co-C bond would be more easily
cleaved. This proposed DMB-dependence mechanism is suggested to may be tested by distinct model systems in future experimental work.

Thus, the study suggests that in an inner-sphere nucleophilic substitution pathway of PCE and TCE with cobalamin, an “on/off” conformational switch-change like similar to “ping-pong” playing is active (see Scheme 4). Specifically, the “base-off” cob(I)alamin facilitates substrate reduction, after which the formed “base-off” chlorinated vinylcobalamins can be readily reduced (the “base-on trichlorovinylcobalamin can be approximately taken as “base off” due to the quite long Co-C bond), then re-coordination of the DMB base provides additional thermodynamic driving force for the heterolytic cleavage of the Co-C bond to complete the overall reaction step.

Scheme 4. The Proposed Reaction Pathway for Cobalamin-Mediated Reductive Dehalogenation of PCE and TCE.
For cob(II)alamin and base-off tri- and di-chlorovinylcob(II)alamin, the spin is mainly localized on Co (spin density from 0.7 to 0.9), so the reactions for cob(II)alamin to cob(I)alamin with spin density from nearly one to zero and tri- and di-chlorovinylcob(III)alamin to tri- and di-chlorovinylcob(II)alamin with the spin density from zero to nearly one, really undergo changes of ~1 imply metal-centered reductions; Mulliken charge of dissociated Cl changes from 0.07 to 0.11 in the reactants, to to (~0.46) to (−0.35) in the transition state, and to (−0.67) ~ (~0.65) in the product complex; Mulliken charge of the CCl2Cl(H) fragment changes from −0.38 to −0.35 in the base-off tri-/di-chlorovinylcob(III)alamin, to −0.70 to −0.66 in the base-off tri-/di-chlorovinylcob(II)alamin, and to −0.71 to −0.44 in the base-on tri-/di-chlorovinylcob(II)alamin respectively (see Supporting Information for more details).

**Inner-Sphere Nucleophilic Addition Mechanism.**

The above work provides the discussion derived the reasonable mechanistic reductive dehalogenation pathway of cobalamin-mediated PCE and TCE. However, the reductive dehalogenation mechanism for cobalamin-mediated less-chlorinated ethylenes, cis-DCE, trans-DCE and VC, is still puzzling. Experimental work has shown that cis-DCE, trans-DCE and VC were reductively dehalogenated by cob(I)alamin in a pH-dependent manner, suggesting that the initial and rate-determining step is possibly the addition of cob(I)alamin to these less-chlorinated ethylenes with simultaneous protonation. Therefore, the nucleophilic addition pathway is computed for cis-DCE, trans-DCE and VC according to eq 5 (taking VC as an example):

\[
\text{Co}^{II} + HCCl + H_2O^+ \rightarrow \text{Co}^{I}HCl + H_2O
\]  \hspace{1cm} (5)

The reaction free energies ($\Delta G_{NA}$) for cob(I)alamin-mediated cis-DCE, trans-DCE and VC during the nucleophilic addition pathway are ~51.9 kcal/mol, ~52.8 kcal/mol and ~48.9 kcal/mol,
respectively. Thus, the notable driving force of the nucleophilic addition pathway for all of the less-chlorinated ethylenes with cob(I)alamin to produce corresponding chlorinated ethylcobalamins is evident.

Subsequently, the $E^0$ and VEA of both the base-on and base-off chlorinated ethylcobalamins were calculated (as is shown in Table 5). Note that the attempts to optimize the one-electron-reduced base-off dichloroethylcobalamin (dichloroethylcob(II)alamin) and chloroethylcobalamin (chloroethylcob(II)alamin) lead directly to the elimination of chloride and formation of VC and ethylene, respectively, so it is not applicable to calculate the $E^0$ values for the base-off less-chlorinated ethylcobalamins. At the same time, the $E^0$ values for the base-on dichloroethylcobalamin and chloroethylcobalamin are $−1.19$ V vs. SCE and $−1.24$ V vs. SCE, respectively, much more negative than the $E^0$ value for the cob(II)alamin/cob(I)alamin couple, i.e. they are difficult to be reduced. However, the VEA values of the base-off forms are larger than the corresponding base-on forms, so it may be inferred that the formed “base-off dichloro- and chloro-ethylcob(II)alamin” would rapidly decompose into the dehalogenation-dehalogenated products. Thus, barring unexpectedly high barriers, the nucleophilic addition with simultaneous protonation for cob(I)alamin-mediated less-chlorinated ethylenes would be favored even in basic solutions with very low concentration of $H_3O^+$.

Experimental work has shown that VC reacted faster with cob(I)alamin than cis-DCE and trans-DCE. The significance of this phenomenon is difficult to evaluate, because it is challenging and error-prone to calculate the acidity constants for transition-metal complexes in solution, thereby and thereby obtain the free energy barriers in the nucleophilic addition pathway with simultaneous protonation is a difficult task. Nevertheless, the calculated proton affinity (PA) for cis-DCE, trans-DCE and VC is $5.4$ eV, $5.5$ eV and $5.8$ eV, respectively, consistent
with the order of the experimental kinetic data (cis-DCE < trans-DCE < VC). Quantitatively, the relationships between the experimental rate constant (log k) at different pH values (pH = 7, 8, and 9) and PA of the less-chlorinated ethylenes reveal significant correlations between both properties (log $k_{\text{pH}=7}$ = 0.22PA + 5.72, $r^2$ = 0.986; log $k_{\text{pH}=8}$ = 0.20PA + 5.87, $r^2$ = 0.994; log $k_{\text{pH}=9}$ = 0.20PA + 6.04, $r^2$ = 0.997). Therefore, PA would be a good probe for the kinetic information in the nucleophilic addition pathway with simultaneous protonation for cob(I)alamin-mediated halogenated compounds.

Table 5. Computed Aqueous-Phase Standard Reduction Potentials ($E^0$) (V, vs. SCE) and Vertical Electron Affinities (VEA) (kcal/mol) for the Base-off and Base-on Forms of Ethylcobalamin

<table>
<thead>
<tr>
<th>Ethynylcobalamin</th>
<th>Base-off E$^0$</th>
<th>VEA</th>
<th>Base-on E$^0$</th>
<th>VEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>dichloroethyl</td>
<td>H $\text{Cl}$ H</td>
<td>70.8</td>
<td>H $\text{Cl}$ H</td>
<td>64.8</td>
</tr>
<tr>
<td>chloroethyl</td>
<td>H $\text{Cl}$ H</td>
<td>65.9</td>
<td>H $\text{Cl}$ H</td>
<td>60.4</td>
</tr>
</tbody>
</table>

In conclusion, the computations provide support for the mechanistic routes and indicate a distinct type of “on/off switch” occurring during cobalamin-mediated reductive dehalogenation of the less-chlorinated ethylenes of the nucleophilic addition pathway: the initial step is the addition of the “base-off” cob(II)alamin to the less-chlorinated ethylenes with simultaneous protonation. Then the formed base-off form of dichloro- and chloro-ethylcobalamin could produce the dehalogenation products directly with formation of “base-on” cob(II)alamin under the reductive reaction conditions (see simplified sketch in Scheme 5).
Scheme 5. The Proposed Reaction Pathway for Cobalamin-Mediated Reductive Dehalogenation of cis-DCE, trans-DCE and VC:

* Mulliken charge of dissociated Cl changes from -0.01 to 0.04 in the reactants, to -0.08 to -0.05 in base-off dichloroethylcob(III)alamin, and to -1.0 in the product complex. Mulliken charge of CH$_2$Cl(H) fragment changes from -0.04 to 0.01 in the reactants, to -0.17 to -0.08 in the base-off tri-/di-chlorovinylcob(III)alamin.s can be found in Supporting Information.

Electronic Structure Characteristics Analysis.

The unique nature of the C−Co−N bonding in cobalamin, with the competing σ and π effects, has continued to be an important mechanistic subject. Figure 5 shows the importantly relevant frontier molecular orbitals during the reductive processes of chlorinated vinylcobalamins, while the relevant frontier molecular orbitals for chlorinated ethylcobalamins are shown in Figure S2 in the Supporting Information. As shown in Figure 5 (a), the lowest unoccupied molecular orbitals (LUMOs) of base-off tri- and di-chlorovinyl cob(III)alamins are largely associated with the σ$_{\text{Co-C}}$, σ$_{\alpha}$ orbital, whereas base-off non-chlorovinyl cob(III)alamins have LUMOs mainly coinciding with the corrin macrocycle π* orbital, and the LUMO of base-off mono-chlorovinyl cob(III)alamin is mixed corrin macrocycle π* (major) and σ$_{\text{Co-C}}$ (minor)−orbital. Interestingly, the mixed
character for the LUMOs of base-off mono-chlorovinyl cob(III)alamin resembles a transition between LUMOs of base-off tri/di-chlorovinyl and non-chlorovinyl cob(III)alamins. The significant difference in LUMO character is mainly due to the fact that the nitrogen atoms of the corrin ring of cobalamin are more electron-rich than the mono- and non-chlorinated ethylene fragments (Mulliken charge: $-0.25 \rightarrow -0.17$) compared with the tri- and di-chlorovinyl ethylene fragments (Mulliken charge: $-0.38 \rightarrow -0.33$), so that the corrin ring has larger overlap with the relevant d-orbital in Co than with the $\text{C}_\alpha$ atom in the ethylene fragment resulting in dominant $\pi^*$ orbital for LUMOs of mono- and non-chlorinated cob(III)alamins ($E_{\text{LUMO}}: -5.05 \rightarrow -5.10$ eV).

By contrast, the strong inductive effect of tri- and di-chlorinated ethylene fragment withdraws electron density from the Co center to reduce the effective nuclear charge of the metal ion, thereby lowering the energies of Co $d_z^2$ orbitals and undergoing a large stabilization of LUMOs on the $\sigma_{\text{Co-C}_\alpha}$ orbital for base-off tri- and di-chlorovinyl cob(III)alamins ($E_{\text{LUMO}}: -5.66 \rightarrow -5.38$ eV).

Generally speaking, the contribution of electron-withdrawing inductive effects from more than or equal to two electronegative chlorine atoms is able to lower the LUMOs of base-off chlorinated vinylcobalamins, thus producing the corresponding lower $E^0$ values shown in Table 4.

These LUMOs of base-off chlorinated vinylcob(III)alamins are initially occupied upon one-electron reduction to produce the corresponding single occupied molecular orbitals (SOMOs). As shown in Figure 5 (b), these SOMOs remain the same as the localization of their precursor LUMOs, as reflected also in the spin densities ($\rho$) of such values are 0.98 $\rightarrow$ 0.99 for SOMOs on the $\sigma_{\text{Co-C}_\alpha}$ orbital of tri- and di-chlorovinyl cob(II)alamins, and 0.87 $\rightarrow$ 0.88 for the corrin macrocycle $\pi^*$ orbital of mono- and non-chlorinated vinylcob(II)alamins. After recoordination of the DMB base, most SOMOs coincide with the corrin
The SOMO localization changes from $\sigma_{\text{Co-Co}}$ orbital-like ($\rho = 0.98$) in the base-off dichlorovinylcobalamin to corrin $\pi^*$ orbital-like ($\rho = 0.88$) in the base-on species. The reason for this major electronic structure change from base-off to base-on dichlorovinylcobalamin is that an additional strong $\sigma$-antibonding between Co and the DMB base in the base-on species leads to a considerable destabilization of the $\sigma_{\text{Co-Co}}$* orbital, thus resulting in SOMO with considerable corrin $\pi$ orbital character. However, the strong withdrawing inductive effect from the three chlorine atoms in base-on trichlorovinylcobalamin is able to cancel out the $\sigma$-donating effect from the DMB base through still reducing the effective nuclear charge of Co atom to lower the energy of its $d_z^2$ orbital, thereby retaining SOMO localization on the $\sigma_{\text{Co-Co}}$* orbital and repelling the DMB base. Interestingly, the distant DMB base in base-on trichlorovinylcobalamin shows weak Van der Waals attraction to further lower the SOMO $\sigma_{\text{Co-Co}}$* orbital, as reflected in comparison of SOMO energies of base-off trichlorovinylcobalamin ($E_{\text{SOMO}} = -2.92$ eV) and base-on trichlorovinylcobalamin ($E_{\text{SOMO}} = -3.07$ eV).
Figure 5. (a) Side View of the LUMOs in the Base-off Chlorinated Vinylcob(III)alamins along with the LUMO Energies ($E_{\text{LUMO}}$); (b) Side View of the SOMOs in the Base-off and Base-on Chlorinated Vinylcob(II)alamins along with the SOMO Energies ($E_{\text{SOMO}}$) as well as Spin Density for Co-C Bond [$p(\text{Co-C})$] and Corrin Macrocycle [$p(\text{Corrin})$].

Isotope Effects.
Table 6 summarizes the calculated average carbon KIE (KIEC) values during the inner-sphere nucleophilic substitution pathway for cobalamin-mediated chloroethylenes, together with the experimental $\varepsilon_{\text{bulkC}}$ as well as the compound average carbon AKIE (AKIEC) obtained from $\varepsilon_{\text{bulkC}}$ according to eq 3. The similarity of the experimental AKIEC values with the computational KIEC values confirm that cobalamin-mediated PCE and TCE dehalogenation proceeds through the nucleophilic substitution pathway. However, the much larger experimental AKIEC values than the vs. KIEC values from for the nucleophilic substitution pathway for cis-DCE and VC, supports the nucleophilic addition of cob(I)alamin to one of the carbon atoms of these chloroethylenes and simultaneous protonation of the other carbon atom, because only the concerted reactions could increase the kinetic isotope effects to the most-full extent.

Table 6. Average Calculated Carbon Kinetic Isotope Effects (KIEC) on the Inner-sphere Nucleophilic Substitution Pathway for Cobalamin-mediated Chloroethylenes, as well as the Experimental Carbon Bulk Isotope Fractionation Factors ($\varepsilon_{\text{bulkC}}$) and the Compound Average Carbon Apparent Kinetic Isotope Effect (AKIEC)

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<tr>
<th></th>
<th>PCE</th>
<th>TCE</th>
<th>cis-DCE</th>
<th>trans-DCE</th>
<th>VC</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIEC</td>
<td>1.028</td>
<td>1.032</td>
<td>1.026</td>
<td>1.030</td>
<td>1.027</td>
</tr>
<tr>
<td>$\varepsilon_{\text{bulkC}}$</td>
<td>$-15.8‰$</td>
<td>$-16.1‰$</td>
<td>$-25.5‰$</td>
<td>/</td>
<td>$-31.1‰$</td>
</tr>
<tr>
<td>AKIEC</td>
<td>1.033</td>
<td>1.033</td>
<td>1.054</td>
<td>/</td>
<td>1.066</td>
</tr>
</tbody>
</table>

Most previous work has focused on stable carbon isotopes to study the transformation process of organic pollutants. However, chlorine also has high-major relevance as a constituent of many polluting environmental compounds. In practice, only a few chlorine isotope analyses have been performed to investigate the transformation of compounds, and the firstly reported chlorine bulk isotope fractionation factor for cobalamin-mediated chloroethylene is $-4.0‰$ for TCE, which
can be converted into AKIECl of ~1.012 by eq 3. The calculated average chlorine KIE for cobalamin-mediated TCE in the inner-sphere nucleophilic substitution pathway is 1.009, quite close to the experimental AKIECl.

As shown in this work, the calculations cannot reproduce very precisely the experimental data, since the latter may be masked. Dual element isotope analysis has attracted considerable interest, the advantage of which is that different mechanisms may be discerned simply by correlating the isotope fractionation factor ($\varepsilon$) ratios for the two elements. Herein, we extend the dual element isotope analysis only on experimental data into a new manner for comparison between computations and experiments through correlating the ratios of $\varepsilon$ or KIE for the two elements. Taking as an example, we consider the reaction of cobalamin-mediated TCE with available dual element isotopes as an example: the reverse eq 3 yields the computational $\varepsilon_C$ of $-15.5\%$ and $\varepsilon_{Cl}$ of $-3.0\%$; thus the ratio of computational $\varepsilon_C$ to $\varepsilon_{Cl}$ in the nucleophilic substitution pathway is calculated to be 5.1 : 1.0, while the ratio of experimental $\varepsilon_C$ ($-16.1\%$) to $\varepsilon_{Cl}$ ($-4.0\%$) is 4.0 : 1.0, so there is some degree of implying some difference between computations and experiments. In the meanwhile, the calculated ratio of AKIEC to AKIECl is 1.02, while the ratio of computational KIEC to KIECl in the nucleophilic substitution pathway is 1.02 as well. Furthermore, the latest reported experimental work concerning combined carbon and chlorine isotope analysis during the reductive dehalogenation of TCE by cobalamin provides a $\varepsilon_C$ value of $-15.0\%$ (AKIE = 1.031) and a $\varepsilon_{Cl}$ value of $-3.2\%$ (AKIE = 1.010), which can be converted into AKIEC/AKIECl of 1.02 again, although there is some difference between the two previous studies for experimental $\varepsilon_C$ and $\varepsilon_{Cl}$ values differ somewhat. Since the most plausible nucleophilic substitution mechanism for cobalamin-mediated TCE is outlined above, the comparison between correlating ratios of KIE for
the two elements may provide a new probe to detect the reaction mechanism. This approach holds promise to bridge a gap between computations and experiments, because various factors mask the AKIEs of both elements to the same extent so that correlating ratios of KIE for the two elements remain the same.

Previous studies have indicated the variability in isotope fractionation of PCE and TCE by a variety of different microorganisms, probably due to the complex enzymatic environment for the dehalogenating strain. Thus, because of this, the elucidation of biological dehalogenation mechanisms may be hampered by the variability in isotope fractionation. In future work, the reaction mechanisms of the biological dehalogenation could be envisioned to be diagnosed potentially by quantum mechanical/molecular mechanical (QM/MM) or cluster modelling to simulate the enzymatic environment in combination with our new proposed method of relating calculated KIE_C/KIE_Cl vs experimental AKIE_C/AKIE_Cl.

Conclusions

This work shows how computational chemistry closes some key unsolved research gaps relating to cobalamin-mediated reduction of chloroethylenes by distinguishing different mechanisms (Scheme 2). To this end, we have developed some useful quantitative methods that rationalize reactivity by (i) serving as screening tools for predicting the reductive dehalogenation reactivity of a given mechanism (e.g., electron affinity for electron transfer, electrophilic index for nucleophilic substitution, and proton affinity for nucleophilic addition); (ii) providing standard reduction potentials \( E^0 \) of formed chlorinated-cobalamins as an important parameter for determining the feasibility of the inner-sphere pathway; (iii) offering suggesting the calculated KIE_C/KIE_Cl vs experimental AKIE_C/AKIE_Cl as a probe for diagnosing the overall reaction mechanism. Thus, these quantitative methods may be useful for in determination of the environmental fate and development of in situ remediation pathways of halogenated organic pollutants.
Recently, Payne et al. proposed a *third type of mechanism*: alternative route, with the cobalamin of reductive dehalogenase able to abstract bromide from 2,6-dibromophenol with formation of a Co–Br bond, consequently leading to a C–Br bond cleavage concomitant with protonation of the leaving group by nearby residue Tyr426. At the same time, Bommer et al. further emphasized the role of strictly conserved Tyr246 in reductive dehalogenase pointing with its phenolic group toward carbon to donate the proton to neutralize the dichlorovinyl anion formed upon reaction of TCE with cobalamin cofactor. The more detailed reaction mechanism of the new proposed dehalogenation mode was computationally elucidated by Liao et al. This new paradigm implies that the repertoire of reductive dehalogenation originating from cobalamin in dehalogenase is even more diverse than previously anticipated, which may guide further modification strategies for cobalamin to mimic the enzyme behavior in *in vitro* reductive dehalogenations. Together with these recent mechanistic findings, our work spans the so far known possible reactivity space of cobalamin in degradation of halogenated compounds, although we expect that further studies are needed to define the relevance of each reaction type to specific conditions and environments.

**ASSOCIATED CONTENT**

**Supporting Information.** Full citation for reference 70; estimation of activation barriers for electron transfer processes by Marcus theory; computed aqueous-phase standard reduction potentials of vinyl radicals; optimized geometries of $S_2$ transition-state of cobalamin-mediated dehalogenation of chloroethylenes; frontier molecular orbitals for less-chlorinated ethylcobalamins; experimental rate constants for reaction of less-chlorinated ethylcobalamin with
cob(I)alamin; energies for all molecular species; Mulliken charges and spin densities; intrinsic reaction coordinate (IRC) for verifying transition states; geometrical comparison between PBE/BSI optimized structures in the gas phase and PBE-D3-PCM/BSI optimized structures; final one-electron symbolic density matrix of complete cob(I)alamin from CASSCF calculation; cartesian coordinates of all molecular structures discussed in this work. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

This work was supported by the National Natural Science Foundation of China (21677125). The China National Supercomputing Center in Shenzhen is acknowledged for providing the Gaussian 09 package and the high-performance computing clusters.
ABBREVIATIONS

DFT, density functional theory; DMB, 5,6-dimethylbenzimidazole; PCE, perchloroethylene; TCE, trichloroethylene; cis-DCE, cis-1,2-dichloroethylene; trans-DCE, trans-1,2-dichloroethylene; VC, vinyl chloride; IRC, intrinsic reaction coordinate; CSIA, compound specific isotope analysis; KIEs, kinetic isotope effects; AKIEs, apparent kinetic isotope effects; PBE, Perdew-Burke-Ernzerhof; ZPE, zero-point energy correction; CPCM, COSMO continuum-solvation model; SCE, standard calomel electrode; VEA, vertical electron affinities; HSAB, hard and soft acids and bases; HOMO, highest occupied molecular orbital; LUMO, lowest unoccupied molecular orbital; LFER, linear free energy relationship; PA, proton affinity.

REFERENCES


(70) Frisch, M. J. et. al., Gaussian 09, revision D.01, Gaussian, Inc.: Wallingford, CT, 2013. see Supporting Information.


**SYNOPSIS**
Electron Transfer vs. Nucleophilic Substitution vs. Nucleophilic Addition

Calculated $\frac{KIE_C}{KIE_A}$ vs. Measured apparent $KIE_C$

apparent $KIE_C$