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Kinetics and mechanism of oxidation of super-reduced cobalamin and cobinamide species by thiosulfate, sulfite and dithionite

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Abstract

We studied the kinetics of reactions of cob(I)alamin and cob(I)inamide with thiosulfate, sulfite, and dithionite by UV-Visible (UV-Vis) and stopped-flow spectroscopy. We found that the two Co(I) species were oxidized by these sulfur-containing compounds to Co(II) forms: oxidation by excess thiosulfate leads to penta-coordinate complexes and oxidation by excess sulfite or dithionite leads to hexa-coordinate Co(II)–SO₂[−] complexes. The net scheme involves transfer of three electrons in the case of oxidation by thiosulfate and one electron for oxidation by sulfite and dithionite. On the basis of kinetic data, the nature of the reactive oxidants was suggested, *i.e.*, HS₂O₃[−] (for oxidation by thiosulfate), S₂O₅^{2−}, HSO₃[−], and aquated SO₂ (for oxidation by sulfite), and S₂O₄^{2−} and SO₂[−] (for oxidation by dithionite). No difference was observed in kinetics with cobalamin(I) or cobinamide(I) as reductants.

Introduction

Cobalamins (Cbls) are ubiquitous natural cofactors of various enzymatic systems and are a required vitamins for higher organisms, including humans. They are complexes of cobalt, existing in +3, +2 and +1 oxidation states, with a corrin tetrapyrrole (an equatorial ligand), a 5,6-dimethylbenzimidazole nucleotide (DMBI, a lower axial ligand), and a variety of upper axial ligands (CH₃[−], 5'-deoxyadenosyl, CN[−] and others). Co³⁺- and Co²⁺-Cbls are found in isomerases (*e.g.*, methylmalonyl-CoA mutase), and Co³⁺- and Co¹⁺-Cbls participate in catalytic cycles of methyltransferases (*e.g.*, methionine synthase) and some other enzymes.^{1–6}

The oxidation state of the cobalt ion in Cbls directly influences the compound's coordination and redox behavior, with a lower cobalt oxidation state decreasing the possible number of

ligands. Co^{3+} -cobalamins (Cbl(III)) exist as six-coordinate complexes, Co^{2+} -cobalamins (Cbl(II)) are predominantly five-coordinated (with the exception of six-coordinate complexes with SO_2^- anion-radical⁷ and thiocyanate)⁸, and Co^{1+} -cobalamin (Cbl(I)) is four-coordinated.³

The transfer of one electron to Cbl(III) occurs readily: the potential of reduction of six-coordinate aquacob(III)alamin to five-coordinate Cbl(II) is -0.04 V vs. standard calomel electrode (SCE) at 22°C .⁹ The product is radicaloid Cbl(II) exhibiting remarkable reactivity in interactions with species bearing uncoupled electrons (*e.g.*, NO ,¹⁰ NO_2 ,¹¹ O_2^- ,¹² SO_2^-).⁷ The transfer of a second electron to cobalamin is a more complex transformation both *in vivo* and *in vitro*. The potential of reduction of base-on Cbl(II) (Cbl with coordinated DMBI) to four-coordinate Cbl(I) is -0.85 V vs. SCE,⁹ which is lower than the potential of all *in vivo* reductants¹³ (including a reductase of methionine synthase).¹⁴ The mechanism of this step is the subject of several publications,^{15–17} with the weakening of axial interactions considered to enhance the potential of the $\text{Co}^{2+} \rightarrow \text{Co}^{1+}$ transformation. The coordination number of cobalt ion in cobalamin-dependent adenosyl transferases approaches the value of four,¹⁶ which probably facilitates Co^{1+} generation in adenosylcobalamin biosynthesis. We showed that the substitution of the relatively tightly bound DMBI in Cbl(II) by a labile water molecule provides for Co(I) formation during reduction by carbohydrates in alkaline solution.^{18,19} In addition, density functional theory (DFT) data predict hydrogen bonding of Cbl(I) to give $\text{Co}^{1+} \cdots \text{H}-\text{X}$ and $\text{X}-\text{H} \cdots \text{Co}^{1+} \cdots \text{H}-\text{X}$ ($\text{X} = \text{OH}$ or imidazole),^{20,21} and an increase in the potential of the $\text{Co}^{2+} \rightarrow \text{Co}^{1+}$ step due to such interactions.²²

Cob(I)alamin is the most potent natural nucleophile known (a so-called “supernucleophile”), and can react with halogenorganics,^{23,24} sucralose,²⁵ epoxides,²⁶ $\text{NO}_2^-/\text{HNO}_2$,²⁷ NH_2OH ,²⁸ $\text{NO}_3^-/\text{HNO}_3$,^{27,29} $\text{ONOO}^-/\text{ONOOH}$,³⁰ N_2O ,³¹ NO ,³² and disulfideorganics.³³ Computations performed by complete active space multiconfigurational perturbation theory (CASPT2)³⁴ and complete active space self-consistent field (CASSCF)³⁵ indicate that a ground state wave function of super-reduced cobalamin is contributed by $\text{Co}^{1+} (\text{d}^8)$ and $\text{Co}^{2+}(\text{d}^7)$ -corrin-radical configurations with substantial predominance of the former. High reactivity of “supernucleophiles” may result from the presence of the biradical component of a ground state electronic configuration, as well as from significant destabilization of the 3d_z^2 orbital of cobalt ion and its facile orientation to contact a substrate.³⁶ Reactivity of supernucleophiles with compounds of the natural sulfur cycle are practically unknown. Reductions of thiosulfate ($\text{S}_2\text{O}_3^{2-}$) and sulfite (SO_3^{2-}) are crucial in this cycle, and, in nature, are catalyzed by thiosulfate-^{37,38} and sulfite-reductases.^{39–51} Several intermediates of these reactions have been elucidated and characterized.^{39–47}

Cob(III)alamin reactions with sulfite^{48–50} and thiosulfate^{50,51} have been studied, as well as $\text{Cbl(III)} \rightarrow \text{Cbl(II)}$ reduction by dithionite ($\text{S}_2\text{O}_4^{2-}$)⁷ and $\text{Cbl(III)} \rightarrow \text{Cbl(II)}$ and $\text{Cbl(II)} \rightarrow \text{Cbl(I)}$ reduction by sulfoxylate (SO_2^{2-}).⁵² $\text{Cbl(II)}-\text{SO}_2^-$ complex formation⁷ and Cbl(I) oxidation by dithionite⁵² have also been demonstrated. Moreover, DFT calculations of several sulfur-containing complexes of Cbl(II) have been published.⁵²

Since cob(I)alamin and thiosulfate and sulfite are abundant in nature and food products, oxidation of Co(I)-complexes by these sulfur compounds likely occurs. This is strongly

supported by the fact that sulfitocob(III)alamin was extracted from mammalian cells.⁵³ Here, we studied the kinetics of oxidation of cob(I)alamin and cob(I)inamide (Cbi(I), an analog of cobalamin lacking the DMBI group) by thiosulfate, sulfite, and dithionite to elucidate the mechanisms of action.

Experimental

Materials

Hydroxocobalamin hydrochloride (HOCbl, 98 %), sodium thiosulfate (98 %), sodium sulfite (98 %), sodium dithionite (86 %), and sodium borohydride (96 %) were obtained from Sigma-Aldrich. Other chemicals used throughout this study were of reagent grade. All solutions were prepared from triple distilled water. Buffer solutions (0.1 M phosphate or borate) were used to maintain a constant pH during experiments. Oxygen-free argon was used to deoxygenate solutions. Solutions of reagents were prepared and handled in gastight glassware. Aquohydroxocobinamide was prepared by base hydrolysis of hydroxocobalamin and purified as previously described.⁵⁴

Cob(I)alamin and cob(I)inamide were prepared by reducing Co(III)-species by sodium borohydride under anoxic conditions.³⁰ Compared to other reductants, for example, Zn (in neutral or acidic media), Ti(III), or sulfoxylate, hydroxymethanesulfinate, or monosaccharides (all in alkaline conditions), borohydride has two advantages: first, excess borohydride can be destroyed by adding acetone, and second, borohydride yields no products possessing redox or binding activity with respect to reagents.

Kinetic measurements

Kinetics of cob(I)alamin and cob(I)inamide oxidation by thiosulfate and sulfite were recorded by monitoring absorption decay at 387 nm. Measurements at pH 8.9 were performed on a cryothermostated ($\pm 0.1^\circ\text{C}$) Cary 50 UV-Vis spectrophotometer, and, at lower pH values, experiments were controlled on a stopped-flow Applied Photophysics Spectra Kinetic spectrophotometer. Kinetics of cob(I)alamin and cob(I)inamide oxidation by dithionite were monitored at 460 nm on a stopped-flow spectrophotometer due to strong dithionite absorption at lower wavelengths. Experimental data were analyzed in Origin 7.5 software.

Results and discussion

Reactions of cob(I)alamin and cob(I)inamide with thiosulfate

Adding excess thiosulfate to cob(I)alamin or cob(I)inamide solutions decreased absorption in the UV-Vis spectrum at 387 nm and at > 550 nm (Figs 1 and S1). UV-Vis spectra of Co(I)-oxidation products at pH 9.4 exhibited maxima at 405 and 475 nm for cobalamin and 469 nm for cobinamide, indicating cob(II)alamin and cob(II)inamide formation according to published data relating to neutral aqueous solutions.⁵⁵ The same products were observed under more acidic conditions. In strongly alkaline media, Co(I) species are stable in the presence of thiosulfate (1×10^{-3} M) for at least 24 h.

Previous workers have shown that Co(I)-corrinoids are gradually oxidized to Co(II) by a proton in weakly alkaline, neutral and acidic solutions.^{27,50,56} We found that cob(I)alamin (Fig. 2) and cob(I)inamide (Fig. S2) were oxidized rapidly by thiosulfate, with kinetic traces well described by single-exponential function. This rapid oxidation suggested little or no contribution by protons, and this behavior remained at lower pH's.

Plots of observed rate constants ($k_{\text{obs}(1)}$) versus the thiosulfate concentration were linear (Fig. 3 and S3), indicating a first order with respect to thiosulfate. The lines intersected the origin in both alkaline and weakly acidic environments (Fig. S4) indicating relatively slow oxidation of Co(I)-complexes by protons under the experimental pH values.

The dependence of $k'_{\text{obs}(1)}$ on pH is shown in Fig. 4. Acidification of solutions significantly enhanced reaction rates, as other investigators found for Cbl(I) oxidation by nitrite, nitrate, and peroxyxynitrite.^{27,30} For the latter three compounds, the increased rate was explained by the increased concentration of protonated oxidant species (nitrous, nitric, and peroxyxynitrous acids, respectively) considered to be reactive compounds in the reaction mechanism. Thiosulfate in acidic media participates in two consecutive proton-binding steps, with pK_a values of 1.74 (pK_{a1}) and 0.6 (pK_{a2}) at 25°C.⁵⁷ Monoprotonated thiosulfate is relatively stable in aqueous solution, whereas the doubly protonated form is unstable decomposing to elemental sulfur, SO_2 and other species.⁵⁸ The reaction rate increase at lower pH can be explained by HS_2O_3^- formation and its interaction with Co(I). Change in redox properties of thiosulfate in acidic solutions has been reported previously.^{59,60}

Increase oxidizing properties of $\text{S}_2\text{O}_3^{2-}$ due to protonation can be explained as follows. First, protonated substrates exhibit more pronounced electrophilic properties as compared to non-protonated ones. Second, protonation decreases electrostatic repulsion between a negatively charged anion and a supernucleophile. Third, protonation can facilitate destabilization of the S–S bond, especially protonation of sulfane sulfur atom.^{61,62}

Kinetic equation (1) was written for the reaction between both cob(I)alamin and cob(I)inamide and $\text{S}_2\text{O}_3^{2-}$. Since $k_{\text{S}_2\text{O}_3^{2-}} \approx 0$, rate constant $k_{\text{HS}_2\text{O}_3^-}$ can be determined using equation (2). Its value was found to be $(6.25 \pm 0.04) \cdot 10^7 \text{ M}^{-1} \text{ s}^{-1}$. In the case of oxidation of Cbl(I), $k_{\text{HS}_2\text{O}_3^-}$ equals to $(5.00 \pm 0.11) \cdot 10^7 \text{ M}^{-1} \text{ s}^{-1}$. The rate constants for these processes are close, which comes probably from the similar structures of cob(I)alamin and cob(I)inamide.

$$\begin{aligned}
 -\frac{d[\text{Co(I)}]}{dt} &= k_{\text{HS}_2\text{O}_3^-} \cdot [\text{Co(I)}] \cdot [\text{HS}_2\text{O}_3^-] \\
 &+ k_{\text{S}_2\text{O}_3^{2-}} \cdot [\text{Co(I)}] \cdot [\text{S}_2\text{O}_3^{2-}] = (k_{\text{HS}_2\text{O}_3^-} \cdot 10^{-\text{pH}} / (10^{-\text{pH}} + 10^{-\text{p}K_{a1}}) + k_{\text{S}_2\text{O}_3^{2-}}) [\text{Co(I)}] \cdot [\text{S}_2\text{O}_3^{2-}] \quad (1)
 \end{aligned}$$

$$k'_{\text{obs}(1)} = k_{\text{HS}_2\text{O}_3^-} \cdot 10^{-\text{pH}} / (10^{-\text{pH}} + 10^{-\text{p}K_{\text{a}1}}) \quad (2)$$

To determine reaction stoichiometry, we performed titration of Cbl(I) with thiosulfate in neutral media (Fig. 5). We found that reduction of one thiosulfate molecule requires three Cbl(I) units, implying a three-electron reduction of thiosulfate (*vide infra*).

The products of both enzymatic^{37,38} and electrochemical⁶³ reduction of thiosulfate are hydrogen sulfide and sulfite, *i.e.*, in the course of reduction the S–S bond cleaves. Indeed, the S–S bond is much longer (2.02 Å)⁶⁴ as compared to the S–O bonds (1.46 Å)⁶⁴. To assess S–S bond cleavage, we used a malachite green probe, since cleavage products (*viz.*, sulfite, sulfide or polysulfide) result in a bleaching solution,⁶⁵ while thiosulfate and polythionates do not give this reaction. Equal concentrations of malachite green were added to a solution of Cbl(I) (6×10^{-5} M) after oxidation by a ten-fold excess of thiosulfate at pH 7.2 and of Cbl(II) mixed with an excess of thiosulfate (a control experiment). In the control experiment, absorbance at 615 nm (absorption maximum of malachite green) was notably higher than in the first experiment (Fig. S5), supporting formation of reduction products of S–S bond in the first experiment.

Formation of H₂S during thiosulfate reduction was demonstrated by UV spectroscopy. Hydrosulfide exhibits maximum absorbance at 230 nm, with an extinction coefficient of $7200 \text{ M}^{-1} \text{ cm}^{-1}$.⁶⁶ After mixing thiosulfate with a 3-fold excess of Cbl(I), we bubbled argon through the solution and collected released gas in anaerobic sodium carbonate (to trap any potential H₂S gas); we found that the UV spectrum of the sodium carbonate was identical to that of HS[−] (Fig. S6).

Reduction of the S–S bond requires two electrons; transfer of a third electron established from stoichiometry is discussed below and occurs from sulfite formation. The rate-determining step may be transfer of the first electron to the HS₂O₃[−] anion leading to formation of the free radical S^{•−} or SO₃^{•−}. Interaction of Co(I) with the free radical proceeds at a substantially higher rate than is apparent from published data.^{23,32} Thus, reactions (3–5) can be written to describe reduction of thiosulfate by Co(I) complexes:

Reactions of cob(I)alamin and cob(I)inamide with sulfite

Addition of sulfite to solutions of cob(I)alamin and cob(I)inamide led to decreased absorption at 387 and > 550 nm, and increased absorption at 460 nm (Fig. 6). Spectra of

products were similar to those of complexes of cob(II)alamin and cob(II)inamide with SO_2^- anion-radical (Figs 6 and S7). The latter can be produced during reactions of Co(III) and Co(II) cobalamin and cobinamide with dithionite,⁷ and during reduction of Co(III) by sulfoxylate⁵² (its one-electron oxidation gives SO_2^-)^{67,68} or hydroxymethanesulfinate ($\text{HOCH}_2\text{SO}_2^-$)⁶⁹ (its solutions generate SO_2^{2-} subsequently oxidizing to SO_2^-)^{67,68}. Formation of this complex readily occurs even at $[\text{Co(II)}] : [\text{S}_2\text{O}_4^{2-}] = 1 : 0.5$; the reaction proceeds extremely rapidly and kinetics cannot be followed using stopped-flow techniques at 5°C. Spectra are typical for these type of complexes and differ substantially from other Co(II) complexes with sulfur-containing ligands: sulfite, thiosulfate, sulfide *etc.* The complex of cob(II)alamin with SO_2^- was found to be six-coordinated with labile DMBI attached to a lower axial site.⁷ Its formation is provided by radical nature of SO_2^- and properties of Cbl(II) as free radical scavenger are well-known.

Kinetic curves of oxidation of both cob(I)alamin and cob(I)inamide (Figs 7 and S8) fit well to a first-order reaction equation. The order does not change at lower pH values, but, in strongly alkaline solutions, oxidation of Co(I)-complexes by sulfite does not proceed.

The dependence of observed rate constants ($k_{\text{obs}(2)}$) of Cbl(I) with sulfite ($[\text{S(IV)}]$) includes a sum of concentrations of SO_3^{2-} , SO_3H^- and SO_2 (Fig. 8a)) is nonlinear, but linearization using the van't Hoff method (Fig. 8 (b)) allows one to determine the reaction order with respect to oxidant ($n = 1.31 \pm 0.02$, pH 9.8, 25°C). The same behavior is observed for cob(I)inamide: the order in sulfite is 1.30 ± 0.03 (Figs S9 and S10, pH 9.7, 25°C). Lowering the pH does not affect the order with respect to oxidant (Fig. S11 and S12, $n = 1.27 \pm 0.03$ at pH 6.4, 25°C (Cbl(I))). The fractional order is usually from several rate-determining steps with the first and second orders with respect to oxidant.

The dependence of $k'_{\text{obs}(2)}$ values on pH can be calculated from equation (6) (Fig. 9). As observed with thiosulfate, the reaction rate increases with lowering of pH. SO_3^{2-} anion can bind two protons to form a mixture of monoprotonated anions (SO_3H^- and HSO_3^- , $\text{p}K_{\text{a}1} = 7.2$, reaction 7), and sulfur dioxide ($\text{SO}_2(\text{aq.})$, $\text{p}K_{\text{a}2} = 1.86$, reaction 8).^{70–73} Apparently one of the oxidants of Co(I) is aqueous sulfur dioxide, since no plateau of pH-dependence at $\text{pH} < \text{p}K_{\text{a}1}$ was found. In more acidic conditions, the reaction rate increases further (data not shown). The concentration of sulfur dioxide is proportional to initial sulfite concentration, indicating a first order in oxidant. A first order in oxidant will be observed also for Co(I) oxidation by bisulfite SO_3H^- . Moreover, the monoprotonated anion of sulfite exists in equilibrium with pyrosulfite ($\text{S}_2\text{O}_5^{2-}$, the constant of dimerization is $K_{\text{d}} = 0.088 \text{ M}^{-1}$, reaction 9).⁷⁴ The concentration of dimer is proportional to the square of the initial sulfite concentration, accounting for the second reaction order in oxidant.

$$k'_{\text{obs}(2)} = k_{\text{obs}(2)} / [\text{S(IV)}]_0^{1.3} \quad (6)$$

To investigate participation of pyrosulfite in the reaction mechanism, we determined reaction orders with respect to oxidant at several temperatures (Fig. 10). Cleavage of the S–S bond consumes heat; therefore, lowering the temperature should increase contribution of pyrosulfite to the reaction, *i.e.*, the order of reaction in oxidant will be enhanced, and *vice versa*. We found that at 12°C $n = 1.48 \pm 0.04$, at 25°C $n = 1.31 \pm 0.02$ and at 39°C $n = 1.01 \pm 0.03$ (pH 9.8), suggesting that $\text{S}_2\text{O}_5^{2-}$ participates in the oxidation of Co(I).

Equation (10) can be expressed for the reaction between Co(I) and S(IV). The equation is based on the assumption that oxidants of Co(I) are SO_2 , SO_3H^- and $\text{S}_2\text{O}_5^{2-}$.

$$\begin{aligned} -d[\text{Co(I)}]/dt &= k_{\text{SO}_2(\text{aq.})} \cdot [\text{Co(I)}] \cdot [\text{SO}_2] \\ &+ k_{\text{S}_2\text{O}_5^{2-}} \cdot [\text{Co(I)}] \cdot [\text{S}_2\text{O}_5^{2-}] + k_{\text{SO}_3\text{H}^-} \cdot [\text{Co(I)}] \cdot [\text{SO}_3\text{H}^-] \\ &= (k_{\text{SO}_2(\text{aq.})} \cdot [\text{S(IV)}] \cdot 10^{(\text{pKa}2-2\text{pH})} / (10^{-\text{pKa}1} + 10^{-\text{pH}}) + k_{\text{S}_2\text{O}_5^{2-}} \cdot [\text{S(IV)}]^2 \cdot K_d \cdot 10^{-2\text{pH}} / (10^{-\text{pKa}1} + 10^{-\text{pH}})^2 \\ &+ k_{\text{SO}_3\text{H}^-} \cdot [\text{S(IV)}] \cdot 10^{-\text{pH}} / (10^{-\text{pKa}1} + 10^{-\text{pH}})) [\text{Co(I)}] \end{aligned} \quad (10)$$

In equation (9) $k_{\text{SO}_2(\text{aq.})}$, $k_{\text{SO}_3\text{H}^-}$ and $k_{\text{S}_2\text{O}_5^{2-}}$ are the reaction rate constants for Co(I) oxidation proceeding *via* formation of SO_2 , SO_3H^- and $\text{S}_2\text{O}_5^{2-}$, respectively; $[\text{S(IV)}]$ is the sum of concentrations of sulfite derivatives as shown in equation (11).

$$[\text{S(IV)}] = [\text{SO}_2] + [\text{SO}_3\text{H}^-] + [\text{SO}_3^{2-}] + [\text{S}_2\text{O}_5^{2-}] \approx [\text{SO}_3\text{H}^-] + [\text{SO}_3^{2-}] \quad (11)$$

To determine rate constants, equation (12) was used. Since analysis of a single concentration dependence cannot distinguish values of $k_{\text{SO}_3\text{H}^-}$ and $k_{\text{SO}_2(\text{aq.})}$, their values were found on the basis of two concentration dependencies obtained at pH 9.82 and 6.4. This analysis yielded values of $(k_{\text{SO}_2(\text{aq.})} \cdot 10^{(\text{pKa}2-\text{pH})} + k_{\text{SO}_3\text{H}^-})$ at each pH, and $k_{\text{SO}_3\text{H}^-}$ and $k_{\text{SO}_2(\text{aq.})}$ were found to be (50.1 ± 3.0) and $(1.26 \pm 0.03)10^8 \text{ M}^{-1} \text{ s}^{-1}$, respectively. Values of $k_{\text{S}_2\text{O}_5^{2-}}$ determined at pH 9.82 and 6.4 were very close: $(4.40 \pm 0.39)10^6$ and $(4.20 \pm 0.10)10^6 \text{ M}^{-1}$.

s^{-1} , respectively. At pH 9.7, $k_{S_2O_5^{2-}}$ for oxidation of cob(I)inamide was $(4.64 \pm 0.24)10^6 M^{-1} s^{-1}$, closely resembling that for cob(I)alamin.

$$k_{obs(2)} = [S(IV)] \cdot \frac{10^{-pH}}{10^{-pKa1} + 10^{-pH}} (k_{SO_2(aq.)} \cdot 10^{(pKa2-pH)} + k_{SO_3H^-}) + k_{S_2O_5^{2-}} \cdot [S(IV)]^2 \cdot K_d \cdot \frac{10^{-2pH}}{(10^{-pKa1} + 10^{-pH})^2} \quad (12)$$

Identity of UV-Vis spectra of final compounds of a process allows one to determine the nature of a product derived during redox reactions.⁷ In the case of sulfite reaction with Co(I), the final product is the radical SO_2^- bound to Co(II). Transformation of superreduced cobalamin and cobinamide in this complex occurs quantitatively. The formation of SO_2^- as a sulfite reduction product is also supported by 1 : 1 stoichiometry (Fig. 11). Therefore, reactions (13–16) express the mechanism of Co(I) oxidation by S(IV)-species.

It is interestingly to compare pathways of sulfite reduction by superreduced cobalamin and cobinamide to that by siroheme-containing sulfite reductase. In the latter case, reaction begins with binding of bisulfite by the Fe(III)-center of siroheme. In subsequent steps (transfer of electrons, protonation, dehydration etc), the sulfite moiety remains coordinated to the iron ion. Formation of relatively stable complexes of sulfite with Co(I) undergoing consecutive acid-base transformations is very unlikely. Reactive oxidant in this mechanism is produced during transformations of free sulfite in solution. Transfer of the first electron in the catalytic cycle of siroheme-containing sulfite reductase occurs to the SO_2 molecule, which exhibits resemblance with processes proceeding with supernucleophiles where an electron is transferred to solvated SO_2 . Moreover, SO_2^- radical was assumed as an intermediate in the catalytic cycle of sulfite reductases.^{47,75} A current study supports its formation during sulfite reduction by metal complexes.⁴⁷

Reactions of cob(I)alamin and cob(I)inamide with dithionite

We reported previously that cob(I)alamin is oxidized in the presence of dithionite in strongly alkaline media.⁵² However, kinetics at pH 13 were affected by a reverse step – reduction of cob(II)alamin by sulfoxylate (the product of reduction of dithionite) back to cob(I)alamin. Since reduction by sulfoxylate proceeds only at pH > 10.5 and oxidation of cob(I)alamin by sulfite (which is obligatorily present in commercial dithionite) occurs at low rates at pH > 9.5, reactions of Co(I)-complexes with dithionite are optimally studied at $9.5 < \text{pH} < 10.5$.

Addition of dithionite to cob(I)alamin and cob(I)inamide resulted in disappearance in the UV-Vis spectrum of an absorption maximum at > 550 nm and increase in a band at ~ 460 nm (Fig. 12 (a)). Absorbance at < 400 nm is caused by dithionite (absorption maximum is at 315 nm). Spectra of products corresponded to those of complexes of Co(II) with anion-radical SO_2^- .

A kinetic trace of the process shown in Fig. 12 (b) fits well to a single-exponential function. The first order in metal complex is observed for oxidation of Cbl(I). In Fig. 13 is shown dependence of the observed rate constants on the dithionite concentration for the reaction with Cbl(I) ($k_{\text{obs}(3)}$). The linear fit of the relationship yields a slope of $(10.5 \pm 0.7) \text{ M}^{-1} \text{ s}^{-1}$ (pH 10). For dithionite reaction with cob(I)inamide, the slope is $(7.8 \pm 0.1) \text{ M}^{-1} \text{ s}^{-1}$ (Fig. S13). Both lines (Fig. 12 and S13) display positive intercepts that result from the oxidation of Co(I).

Oxidation of Co(I) complexes is feasible by two species – dithionite (reactions 17 and 18) and SO_2^- anion-radical (reaction 19) (the monomerization constant of dithionite (reaction 20) is $K_m = 1.4 \times 10^{-9} \text{ M}$ at 25°C)⁷⁶. For the latter condition, equation 21 shows expression of the observed rate constant. Reduction of the concentration dependencies (Fig. 13 and S13) to a $k_{\text{obs}(3)}/[\text{SO}_2^-]$ vs. $[\text{SO}_2^-]$ form allows calculation of rate constants (Table 1).

$$k_{\text{obs}(3)} = k_{\text{SO}_2^-}^1 \cdot [\text{SO}_2^-] + k_{\text{S}_2\text{O}_4^{2-}}^1 \cdot [\text{S}_2\text{O}_4^{2-}] = k_{\text{SO}_2^-}^1 \cdot [\text{SO}_2^-] + k_{\text{S}_2\text{O}_4^{2-}}^1 \cdot [\text{SO}_2^-]^2 / K_m \quad (21)$$

Titration of Cbl(I) by dithionite allows one to determine reaction stoichiometry (Fig. 14), and we found $[\text{Cbl(I)}] : [\text{S}_2\text{O}_4^{2-}] = 1:0.9$. Thus, the predominant pathway of oxidation of Co(I) complexes was by reactions (17) and (18) from relatively low concentrations of SO_2^- in the system.

Chemical reduction of dithionite is an extremely challenging task because of dithionite's strong reducing properties. Besides the reactions with cob(I)alamin and cob(I)inamide, the only example of dithionite reduction by metal complexes is that dithionite oxidizes Mo(II) to Mo(V) in a $(\text{C}_5\text{R}_5)\text{Mo}(\text{CO})_3\text{H}$ ($\text{X} = \text{H}, \text{Me}$) complex.⁷⁷

The product of Cbl(I) reduction of dithionite and SO_2^- anion-radical is sulfoxylate (SO_2^{2-}). Its protonation results in formation of SO_2H^- ⁷⁸ and $\text{S}(\text{OH})_2$ species⁷⁹. Only the SO_2^{2-} anion can reduce Co(II) to Co(I)⁵², since reaction of Co(II) with sulfoxylate proceeds at $\text{pH} > 10.5$. Moreover, fitting the pH-dependence of cobinamide(II) reduction by sulfoxylate to a sigmoidal function produces $\text{p}K_a$ similar to $\text{p}K_a$ of deprotonation of SO_2H^- anion (SI, 13.6 at 25°C)⁷⁹. Observed rate constants of reactions (22) and (23) are in Table 1 (for cobalamin and cobinamide, kinetics is reported in ⁵² and SI, respectively). Hence, the reactions (24) and (25) are reversible in strongly alkaline media, and macroscopic equilibrium constants can be calculated as the quotient of rate constants of forward and reverse steps. Equilibrium constants for reactions (24) and (25) can be determined using equation (26).

$$K_{\text{Co(I)/Co(II)}} = \frac{k_{\text{SO}_2^- \text{ (or } \text{S}_2\text{O}_4^{2-})}^1}{k_{\text{SO}_2^- \text{ (or } \text{S}_2\text{O}_4^{2-})}^{-1} (10^{\text{p}K_a(\text{SO}_2\text{H}^-) - \text{pH}} + 1)} \quad (26)$$

Conclusions

This work showed that superreduced species of cobalamin and cobinamide efficiently reduce thiosulfate to sulfite and hydrogen sulfide, and sulfite to anion-radical of sulfur dioxide. We propose that reduction of thiosulfate proceeds *via* formation of its monoprotonated form and reduction of sulfite *via* formation of sulfur dioxide, bisulfite, and pyrosulfite. We suggested the mechanism of Co(I) oxidation by dithionite including both $\text{S}_2\text{O}_4^{2-}$ and SO_2^- species. This work has important implications in terms of potential reduction of thiosulfate and sulfite in foods supplemented with cobalamin (vitamin B₁₂), particularly given the frequent use of sulfite as a preservative, as well as in nature.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.
UV-Vis spectral changes recorded for Cbl(I) oxidation by thiosulfate. $[\text{Cbl}] = 6 \times 10^{-5} \text{ M}$;
 $[\text{S}_2\text{O}_3^{2-}] = 1 \times 10^{-3} \text{ M}$; pH 9.4; 25°C .

Fig. 2.
Typical kinetic curve of oxidation of Cbl(I) (5×10^{-5} M) by thiosulfate (2×10^{-3} M) at pH 9.4, 25°C.

Fig. 3.
Plot of $k_{obs(1)}$ vs. $[S_2O_3^{2-}]$ for oxidation of Cbl(I) ($5 \times 10^{-5} M$) by at least of 20-fold thiosulfate excess over complex at pH 9.08, 25°C.

Fig. 4.

Plot of $k'_{\text{obs}(1)}$ versus pH for oxidation of Cbl(I) (5×10^{-5} M) by at least of 20-fold thiosulfate excess over complex at 25°C with best fit to equation (2).

Fig. 5. UV-Vis spectra recorded during titration of Cbl(I) (2.9×10^{-4} M) by thiosulfate at pH 6.5, 25°C. Inset: dependence of absorbance at 684 nm on initial thiosulfate concentration.

Fig. 6.
UV-Vis spectral changes during Cbl(I) oxidation by sulfite. $[\text{Cbl}] = 6 \times 10^{-5} \text{ M}$; $[\text{SO}_3^{2-}] = 1 \times 10^{-2} \text{ M}$; pH 9.4; 25°C.

Fig. 7.
Typical kinetic curve of oxidation of Cbl(I) (5×10^{-5} M) by sulfite (2×10^{-2} M) at pH 9.8, 25°C.

Fig. 8. Plots of $k_{\text{obs}(2)}$ vs. [S(IV)] (a) and $\ln(k_{\text{obs}(2)})$ vs. $\ln([S(IV)])$ (b) for oxidation of Cbl(I) (5×10^{-5} M) by at least of 180-fold sulfite excess over complex at pH 9.8, 25°C.

Fig. 9.
Plot of k'_{obs} vs. pH for reaction of Cbl(I) ($5 \times 10^{-5} \text{ M}$) with sulfite 25°C .

Fig. 10.
Plots of k_{obs} vs. $[\text{S(IV)}]$ for the reaction of Cbl(I) ($5 \cdot 10^{-5}$ M) with a sulfite at 12 (1), 25 (2) and 39 (3) °C, pH 9.8.

Fig. 11. UV-Vis spectra recorded during the titration of Cbl(I) (3.3×10^{-4} M) by sulfite at pH 7, 25°C. Inset: plot of absorbance at 684 vs. initial sulfite concentration.

Fig. 12.

UV-Vis spectra of 2×10^{-5} M Cbl(I) (solid line) and of the product of its oxidation by 0.001 M dithionite (dashed line) (a), and a typical kinetic curve of oxidation of Cbl(I) (7×10^{-5} M) by dithionite (6×10^{-4} M) at pH 10, 25°C (b).

Fig. 13.
Plot of $k_{\text{obs}(3)}$ vs. $[\text{S}_2\text{O}_4^{2-}]$ for the reaction between Cbl(I) ($7 \times 10^{-5} \text{ M}$) and dithionite at pH 10, 25°C .

Fig. 14. UV-Vis spectra during titration of Cbl(I) (3.6×10^{-4} M) by dithionite at pH 10.5, 25°C. Inset: plot of absorbance at 684 nm vs. initial dithionite concentration.

Table 1Characteristics of reactions of Co(I) and Co(II) with $\text{S}_2\text{O}_4^{2-}$ (SO_2^-) and SO_2^{2-}

Constants	Complex	
	Cobalamin	Cobinamide
$k^1_{\text{S}_2\text{O}_4^{2-}}/M^{-1} \text{ s}^{-1}$	4.90 ± 0.45	6.13 ± 0.31
$k^1_{\text{SO}_2^-}/M^{-1} \text{ s}^{-1}$	$(1.35 \pm 0.06)10^4$	$(2.82 \pm 0.29)10^3$
$k^{-1}_{\text{S}_2\text{O}_4^{2-}}/M^{-1} \text{ s}^{-1}$	2.83 ± 0.11	2.18 ± 0.07
$k^{-1}_{\text{SO}_2^-}/M^{-1} \text{ s}^{-1}$	5.92 ± 0.08	3.30 ± 0.11
$K_{\text{Co(I)/Co(II)}}(\text{S}_2\text{O}_4^{2-})^c/M^{-1}$	0.35 ± 0.05	0.57 ± 0.05
$K_{\text{Co(I)/Co(II)}}(\text{SO}_2^-)^c/M^{-1}$	458.2 ± 26.6	172.4 ± 23.4

^a determined at 25°C, pH 10 (0.1 M borate buffer).^b observed values determined at 25°C, pH 13 (0.1 M NaOH).^c determined as a quotient of k^1 and k^{-1} .