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Interaction of Cob(III)alamins with Hypothiocyanite. Evidence for the Formation of Hypothiocyanitocobalamin

Ilia A. Dereven'kov,@ and Vladimir S. Osokin

Ivanovo State University of Chemistry and Technology, 153000 Ivanovo, Russia
@Corresponding author E-mail: derevenkov@gmail.com

Here, we report the results of investigation of the reactions between cobalamins (Cbls) and hypothiocyanite ($OSCN^-$), a product of thiocyanate oxidation by hydrogen peroxide in the presence of lactoperoxidase. In the case of aquacobalamin, the product, the UV-vis spectrum of which differs from the UV-vis spectra of Cbl(III)-complexes with SCN^-, SO_3^{2-}, OCN^- and CN^- anions, is formed and attributed to hypothiocyanitocobalamin. This complex reacts with selenomethionine at substantially lower rate than the free $OSCN^-$. Cyano- and methyl-Cbls are relatively stable in the presence of $OSCN^-$. Glutathionylcobalamin is transformed to hypothiocyanitocobalamin in the presence of $OSCN^-$ excess via the oxidation of glutathionyl-ligand and subsequent binding of $OSCN^-$ by Cbl(III).

Keywords: Cobalamins, hypothiocyanite, lactoperoxidase, thiocyanate, coordination.

Взаимодействие Co(III)−форм кобаламинов с гипотиоцианитом. Подтверждение образования гипотиоцианитокобаламина

И. А. Деревеньков,@ В. С. Осокин

Ивановский государственный химико-технологический университет, 153000 Иваново, Россия
@E-mail: derevenkov@gmail.com

Представлены результаты исследования реакций кобаламинов (Cbls) и гипотиоцианитом ($OSCN^-$), образующимся в результате окисления тиоцианата пероксидом водорода в присутствии лактопероксидазы. При взаимодействии аквакобаламина с $OSCN^-$ образуется продукт, который имел электронный спектр поглощения (ЭСП), отличающийся от ЭСП комплексов Cbl(III) с SCN^-, OCN^-, SO_3^{2-} и CN^-, и был отнесен к гипотиоцианитокобаламину. Этот комплекс реагирует с селенометионином значительно медленнее, чем свободный гипотиоцианит. Показано, что циано- и метилкобаламины устойчивы в присутствии $OSCN^-$. Глутатионилкобаламин переходит в гипотиоцианитокобаламин в присутствии избытка $OSCN^-$ через окисление глутатионильного лиганда и последующее связывание $OSCN^-$ Co(III)-кобаламином.

Ключевые слова: Кобаламины, гипотиоцианит, лактопероксидаза, тиоцианат, координация.

Hypothiocyanite ($OSCN^-$) is the product of thiocyanate (SCN^-) oxidation by hydrogen peroxide mediated by peroxidases (*i.e*., lacto-, myelo- and eosinophile peroxidases).[1] Reactions between SCN^- with several other oxidants (*i.e*., hypochlorite,[2a] hypobromite,[2b] chloramine,[2c] peroxomonosulfate[2d]) lead to $OSCN^-$ as well. $OSCN^-$ is an important component of immune system damaging the pathogen cells.[3] However, several negative effects on human health are associated with $OSCN^-$.[4] In contrast to the other products generated by above-mentioned peroxidases (*viz*., hypochlorite and hypobromite), it is a relatively soft oxidant predominantly reacting with thiol groups.[5] Reactions of $OSCN^-$ with selenium species (*e.g.,* selenols and selenomethionine),[6] tryptophane,[7] and other substrates have been reported as well. Several works analyze the stability of $OSCN^-$.[8] However, there is a little attention paid to the coordination properties of $OSCN^-$. Only transient formation of $OSCN^-$ bound to ruthenium(III) ion

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was suggested in the course of SCN^- oxidation by H_2O_2 or peroxomonosulfate in the presence of Ru(III)(edta) complex.[9]

Cobalamins (Cbls) are the ubiquitous cobalt corrin complexes.[10] Cbls are cofactors for methionine synthase and methylmalonyl-CoA mutase.[11] Behavior of Cbls in the catalysis[12] and medicine[13] has been thoroughly highlighted as well. Co(III)-ion in aquacobalamin (H_2OCbl) is capable of binding various anionic and neutral ligands.[14] For example, Cbl(III) forms a tight complex with CN^- ($K = 1{\cdot}10^{12}$ M^{-1})[15] and much weaker complexes with SCN^- ($K = 1.1{\cdot}10^3$ M^{-1} at 25.0 °C, $I = 2.2$ M)[16] and OCN^- ($K = 3.7{\cdot}10^2$ M^{-1} at 25.0 °C, $I = 2.2$ M).[16a] In this work, we have examined complexation of $OSCN^-$ with H_2OCbl in weakly acidic medium.

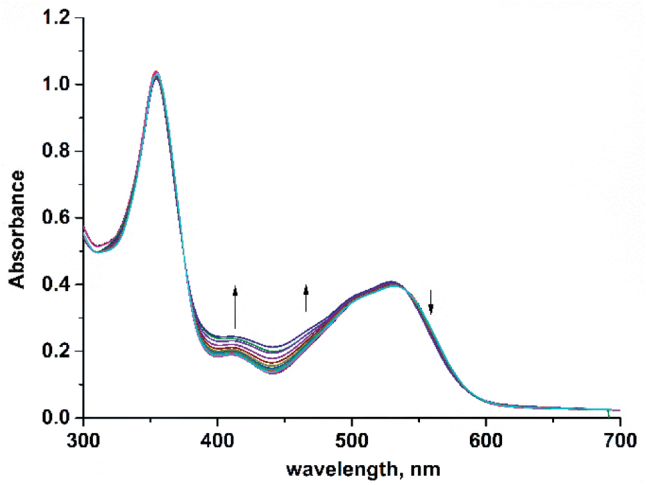

Figure 1. UV-vis spectra collected after mixing of H_2OCbl ($5.0{\cdot}10^{-5}$ M) with LPO (*ca.* 0.6 U/mL), SCN^- (2.0 mM) and H_2O_2 (2.0 mM) at p*H* 6.5 and 25.0 °C. Mixture of LPO (*ca*. 0.6 U/mL), SCN^- (2.0 mM) and H_2O_2 (2.0 mM) at p*H* 6.5 generates $[OSCN^-] = 1.0$ mM. Time interval between spectra is 1 min. The initial spectrum corresponds to thiocyanatocobalamin, the final spectrum – to new Cbl(III) complex.

The addition of H_2OCbl to the mixture of lactoperoxidase (LPO), SCN^- and H_2O_2 results in rapid formation of thiocyanatocobalamin and further slower reaction illustrated by UV-vis spectral changes (Figure 1), *i.e.* an increase in absorbance is observed between 375–540 nm. These observations cannot be explained by the absorbance of free $OSCN^-$, which exhibits very weak peak at 376 nm (extinction coefficient is 26.5 $M^{-1}{\cdot}cm^{-1}$) and negligibly absorbs at 410–540 nm (Figure S1 of Supporting Information).[17] The products of $OSCN^-$ decomposition (*viz*., OCN^-, SO_3^{2-} and CN^-)[8c,17] are capable of binding to Cbl(III), however, their UV-vis spectra (Figure 2) do not support formation of cyano-, sulfito- and cyanatocobalamins in the H_2OCbl/LPO/SCN^-/H_2O_2 system. Similar UV-vis spectral changes were obtained upon mixing of H_2OCbl with $OSCN^-$/SCN^- mixture prepared via basic hydrolysis of thiocyanogen ($(SCN)_2$; Figure S2).[8a,17] UV-vis spectral changes illustrated in Figure 1 are different from those observed for H_2OCbl/LPO/H_2O_2 system in the absence of SCN^- (Figure S3): even in the presence of high LPO quantities, slow H_2OCbl degradation is observed. Thus, we attribute the spectra shown in Figure 1 to the formation of hypothiocyanitocobalamin.

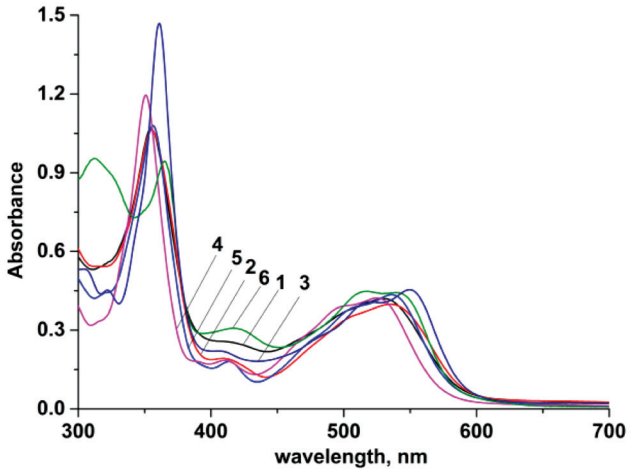

Figure 2. UV-vis spectra of the product of the reaction between H_2OCbl and the mixture of LPO (*ca*. 0.6 U/mL), SCN^- (5.0 mM) and H_2O_2 (1.0 mM; 1), thiocyanatocobalamin (2), cyanocobalamin (3), H_2OCbl (4), cyanatocobalamin (5) and sulfitocobalamin (6). $[Cbls] = 5{\cdot}10^{-5}$ M; p*H* 6.5; 25.0 °C. Mixture of LPO (*ca*. 0.6 U/mL), SCN^- (5.0 mM) and H_2O_2 (1.0 mM) at p*H* 6.5 generates $[OSCN^-] = 0.9$ mM.

Next, behavior of the other Cbl species in LPO/SCN^-/H_2O_2 system was examined. Cyano- (CNCbl) and methylcobalamins (MeCbl), which contain tightly bound ligands in the upper axial position, do not react with species derived from LPO/SCN^-/H_2O_2 system (Figures S4 and S5). Glutathionylcobalamin (GSCbl), another tight Cbl(III)-complex, undergoes transformation in LPO/SCN^-/H_2O_2 system to hypothiocyanitocobalamin (Figure S6), probably, via oxidation of glutathione motif by $OSCN^-$, since thiol group is highly reactive toward $OSCN^-$.[5] However, substitution of glutathionyl-ligand by $OSCN^-$ with subsequent oxidation of free glutathione (GSH) by $OSCN^-$ cannot be excluded as well.

To determine equilibrium constant for hypothiocyanitocobalamin formation (K_1), titration of thiocyanatocobalamin by $OSCN^-$ in the presence of constant SCN^- concentration (0.01 M) was performed (Figure S7). Using eq. (S2), $K_1 = (1.1 \pm 0.2){\cdot}10^4$ M^{-1} (p*H* 6.5; 25.0 °C) was calculated. This value is *ca.* 10-fold higher than equilibrium constant for thiocyanatocobalamin formation.[16a] Hypothiocyanitocobalamin is relatively unstable and undergoes decomposition to thiocyanatocobalamin (Figure 3) with the rate constant $k = (1.2 \pm 0.1){\cdot}10^{-3}$ s^{-1} (p*H* 6.3; 25.0 °C; $[SCN^-] = 10.0$ mM).

$OSCN^-$ is reactive toward selenomethionine (Sem) with a rate constant of $2.8{\cdot}10^3$ $M^{-1}{\cdot}s^{-1}$ (p*H* 7.4; 25.0 °C).[6] We examined how $OSCN^-$ binding to Cbl(III) affects its oxidizing properties using Sem as poorly coordinating ligand to Cbl(III) (*i.e*., Sem does not bind Cbl(III) in applied concentration range; Figure S8). Reaction of hypothiocyanitocobalamin with Sem conducted under the excess of SCN^- produces thiocyanatocobalamin and is characterized by the first

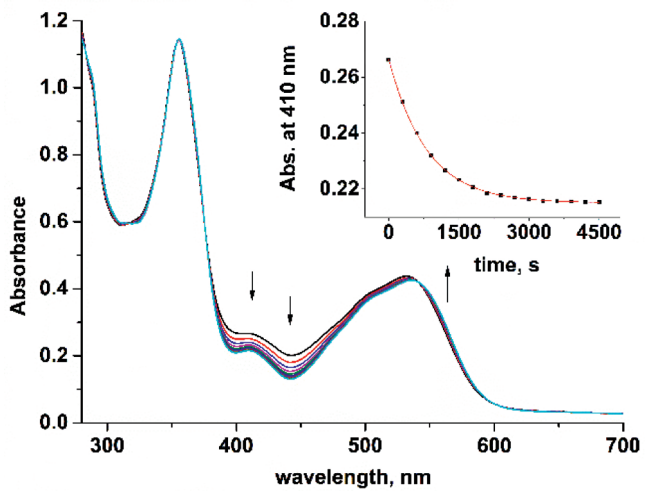

Figure 3. UV-vis spectra collected during decomposition of hypothiocyanitocobalamin ($5.0 \cdot 10^{-5}$ M) at p*H* 6.3, 25.0 °C, $[OSCN^-]_0$ = 0.8 mM. Insert: kinetic curve of the reaction.

is transformed to hypothiocyanitocobalamin, probably, via oxidation of glutathionyl-ligand by $OSCN^-$.

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References

1. a) Nagy P., Alguindigue S.S., Ashby M.T. *Biochemistry* **2006**, *45*, 12610–12616 (doi: 10.1021/bi061015y); b) Furtmüller P.G., Jantschko W., Regelsberger G., Jakopitsch G., Arnhold J., Obinger C. *Biochemistry* **2002**, *41*, 11895–11900 (doi: 10.1021/bi026326x); c) van Dalen C.J., Whitehouse M.W., Winterbourn C.C., Kettle A.J. *Biochem. J.* **1997**, *327*, 487–492 (doi: 10.1042/bj3270487); d) Slungaard A., Mahoney J.R. *J. Biol. Chem.* **1991**, *266*, 4903–4910; e) Schlorke D., Flemmig J., Gaua J., Furtmüller P.G., Obinger C., Arnhold J. *J. Inorg. Biochem.* **2016**, *162*, 117–126 (doi: 10.1016/j.jinorgbio.2016.06.019).
2. a) Ashby M.T., Carlson A.C., Scott M.J. *J. Am. Chem. Soc.* **2004**, *126*, 15976–15977 (doi: 10.1021/ja0438361); b) Nagy P., Beal J.L., Ashby M.T. *Chem. Res. Toxicol.* **2006**, *19*, 587–593 (doi: 10.1021/tx050338c); c) Xulu B.A., Ashby M.T. *Biochemistry* **2010**, *49*, 2068–2074 (doi: 10.1021/bi902089w); d) Kalmár J., Lente G., Fábián I. *Inorg. Chem.* **2013**, *52*, 2150–2156 (doi: 10.1021/ic302544y).
3. Hawkins C.L. *Free Radic. Res.* **2009**, *43*, 1147–1158 (doi: 10.3109/10715760903214462).
4. Barrett T.J., Hawkins C.L. *Chem. Res. Toxicol.* **2012**, *25*, 263–273 (doi: 10.1021/tx200219s).
5. a) Barrett T.J., Pattison D.I., Leonard S.E., Carroll K.S., Davies M.J., Hawkins C.L. *Free Radic. Biol. Med.* **2012**, *52*, 1075–1085 (doi: 10.1016/j.freeradbiomed.2011.12.024); b) Skaff O., Pattison D.I., Davies M.J. *Biochem. J.* **2009**, *422*, 111–117 (doi: 10.1042/BJ20090276); c) Nagy P., Jameson G.N., Winterbourn C.C. *Chem. Res. Toxicol.* **2009**, *22*, 1833–1840 (doi: 10.1021/tx900249d).
6. Skaff O., Pattison D.I., Morgan P.E., Bachana R., Jain V.K., Priyadarsini K.I., Davies M.J. *Biochem. J.* **2012**, *441*, 305–316 (doi: 10.1042/BJ20101762).

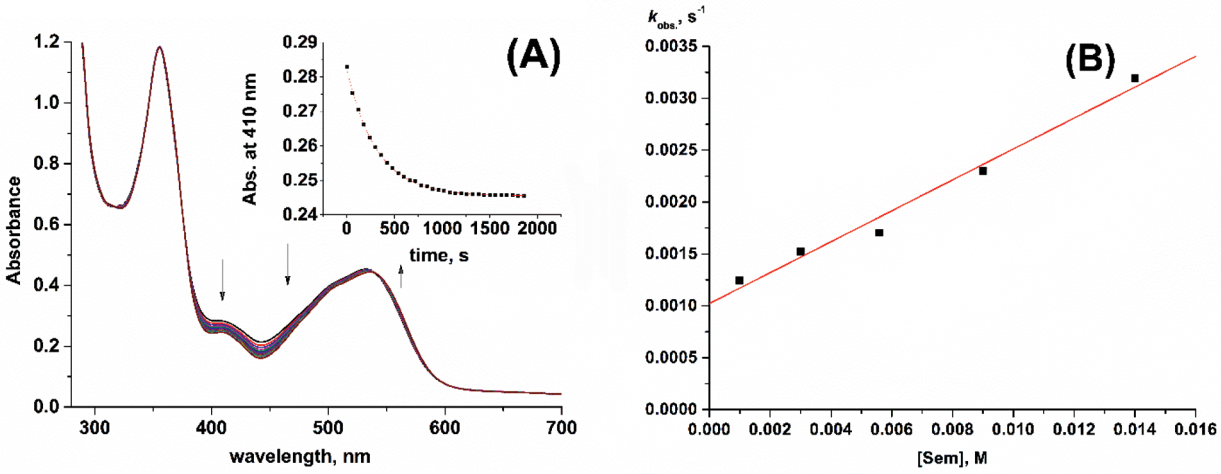

Figure 4. UV-vis spectra of the reaction between hypothiocyanitocobalamin ($5.0 \cdot 10^{-5}$ M) and selenomethionine (Sem; $1.4 \cdot 10^{-2}$ M; A) and the plot of observed rate constant ($k_{obs.}$) versus [Sem] (B) at p*H* 6.3, 25.0 °C, $[OSCN^-]_0$ = 0.8 mM, $[SCN^-]$ = 10.0 mM. Insert: kinetic curve of the reaction between hypothiocyanitocobalamin ($5.0 \cdot 10^{-5}$ M) and Sem ($1.4 \cdot 10^{-2}$ M).

order with respect to hypothiocyanitocobalamin (Figure 4). The dependence of the observed rate constant on [Sem] exhibits the positive intercept, which value coincides with a rate constant of hypothiocyanitocobalamin decomposition under given conditions (vide supra). The value of the slope is $(1.5 \pm 0.2) \cdot 10^{-1}$ $M^{-1} \cdot s^{-1}$, which is substantially lower than the rate constants for the reactions of free $OSCN^-$ with Sem. Thus, binding to Cbl(III) substantially decreases the reactivity of $OSCN^-$.

In this work, we provided an evidence for the formation of the complex between Cbl(III) and the product generated in LPO/SCN^-/H_2O_2 system, the UV-vis spectrum of which differs from that of Cbl(III)-complexes with SCN^-, OCN^- or CN^-. This product was attributed to hypothiocyanitocobalamin. It exhibits oxidizing properties toward Sem, although it is substantially less reactive than free $OSCN^-$. CNCbl and MeCbl are inert toward $OSCN^-$, whereas GSCbl

7. Hawkins C.L., Pattison D.I., Stanley N.R., Davies M.J. *Biochem. J.* **2008**, *416*, 441–452 (doi: 10.1042/BJ20070941).
8. a) Nagy P., Wang X., Lemma K., Ashby M.T. *J. Am. Chem. Soc*. **2007**, *129*, 15756–15757 (doi: 10.1021/ja0770532); b) Nagy P., Lemma K., Ashby M.T. *Inorg. Chem.* **2007**, *46*, 285–292 (doi: 10.1021/ic061470i); c) Kalmár J., Woldegiorgis K.L., Biri B., Ashby M.T. *J. Am. Chem. Soc*. **2011**, *133*, 19911–19921 (doi: 10.1021/ja2083152).
9. a) Chatterjee D., Paul B., Mukherjee R. *Dalton Trans*. **2013**, *42*, 10056–10060 (doi: 10.1039/c3dt50723h); b) Sarkar P., Chatterjee D. *Int. J. Chem. Kin*. **2016**, *48*, 117–123 (doi: 10.1002/kin.20976).
10. a) Brown K.L. *Chem. Rev*. **2005**, *105*, 2075–2149 (doi: 10.1021/cr030720z); b) Dereven'kov I.A., Salnikov D.S., Silaghi-Dumitrescu R., Makarov S.V., Koifman O.I. *Coord. Chem. Rev*. **2016**, *309*, 68–83 (doi: 10.1016/j.ccr.2015.11.001).
11. Kräutler B. *Biochem. Soc. Trans*. **2005**, *33*, 806–810 (doi: 10.1042/BST0330806).
12. Giedyk M., Goliszewska K., Gryko D. *Chem. Soc. Rev*. **2015**, *44*, 3391–3404 (doi: 10.1039/C5CS00165J).
13. Zelder F. *Chem. Commun*. **2015**, *51*, 14004–14017 (doi: 10.1039/C5CC04843E).
14. Marques H.M., Knapton L. *J. Chem. Soc., Dalton Trans*. **1997**, 3827–3833 (doi: 10.1039/A703139D).
15. Hayward G.C., Hill H.A.O., Pratt J.M., Vanston N.J., Williams R.J.O. *J. Chem. Soc*. **1965**, 6485–6493 (doi: 10.1039/JR9650006485).
16. a) Knapton L., Marques H.M. *Dalton Trans*. **2005**, 889–895 (doi: 10.1039/B416083E); b) Garau G., Geremia S., Marzilli L.G., Nardin G., Randaccio L., Tauzher G. *Acta Cryst*. **2003**, *B59*, 51–59 (doi: 10.1107/S0108768102019353); c) Dereven'kov I.A., Salnikov D.S., Makarov S.V., Surducan M., Silaghi-Dumitrescu R., Boss G.R. *J. Inorg. Biochem*. **2013**, *125*, 32–39 (doi: 10.1016/j.jinorgbio.2013.04.011).
17. Ashby M.T. *Adv. Inorg. Chem*. **2012**, *64*, 263–303 (doi: 10.1016/B978-0-12-396462-5.00008-8).

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