

Ligand binding properties of cobalamins

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Abstract The main goal of the present density functional theory calculations is a comparative study of NO, O₂, NO₂⁻, and H₂O binding to different forms of cob(II)alamins and cob(III)alamins. The comparison of binding energies of small ligands enables one to draw conclusions regarding the stability of the studied derivatives of cobalamins as well as to define the preferred form of cobalamin for each ligand. Ligands such as NO and O₂ favor cob(II)alamins, while H₂O and NO₂⁻ cob(III)alamins. The obtained results are confronted with available experimental data. Finally, our findings allow one to divide the studied small ligands into two groups: NO and O₂ for which the coordination to cobalamins significantly weakens their internal bonds, and NO₂⁻ and H₂O for which the effect is not observed.

Keywords Bioinorganic chemistry · Cobalamins · Density functional calculations · Ligand binding · Nitrogen oxides

1 Introduction

Cobalamins are the group of coordination compounds in which cobalt ion, adopting oxidation states ranging from +1 to +3, is coordinated equatorially by the corrin ring. One of

the axial ligand is the dimethylbenzimidazole molecule, the second is different depending on the derivative of cobalamin. The significance of this class of complex molecules follows a fact that their presence is necessary for metabolism. Further, they play a key role in numerous important processes such as 1,2-intramolecular rearrangements, reductions of ribonucleotides to deoxyribonucleotides, methylation of homocysteine to methionine and CO₂ fixation [1].

The most studied cobalamin complexes are AdoCbl (adenosylcobalamin) and CH₃Cbl (methylcobalamin), in which the cobalt ion forms a bond with carbon atom. So far, many experimental techniques were used to investigate the formation and stability of their different forms. Advanced theoretical methods were also employed to study these derivatives (see for example [2–12]). In addition, cobalamin derivatives with such inorganic ligands present in biological environment as H₂O, NO, NO₂⁻ and O₂ are known [13–18]. Among them, nitrosyl complexes are probably the most experimentally investigated, due to their great importance in the mediation of physiological effects that depend on nitric oxide (NO). It is postulated that cobalamins may act as scavengers of NO during septic shock when amounts of released NO become so large that may be harmful for the organism [19, 20]. One of the main questions that arises concerns the form of cobalamin that effectively binds NO in water solution; is it cob(II)alamin or cob(III)alamin? Despite the importance of the issue, up to our knowledge, it is investigated mainly only by experimental techniques, whereas the theoretical studies of the above listed inorganic derivatives of cobalamins are limited to six-coordinate nitrosyl and aqua complexes of both cob(II)- and cob(III)alamin [21] as well as to complexes for which structural data are available [22]. There is still a lack of theoretical investigation of five-coordinate nitrosyl and aqua complexes and any of O₂ and NO₂⁻ derivatives of complexes in question. In [21] the different levels of theory were employed

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ranging from wave function methods (HF, MP2 and ROMP2) to DFT-based approaches where hybrid B3LYP and non-hybrid PW91 functionals were used. The reported results seem to favor the hypothesis that it is a cob(II)alamin that binds NO rather than cob(III)alamin, however, the obtained results were largely dependent on the method applied, especially regarding ligand binding energies. A conclusion that one should use non-hybrid functionals within DFT approach to study these type of adducts was made.

The significance of the comparative study of cob(II)- and cob(III)alamin complexes with NO, H₂O, NO₂⁻ and O₂ ligands may be explained as follows. It should be borne in mind that in aerobic conditions, which are found in vivo as well as for the majority of the experiments in vitro, water solution of NO contains NO₂⁻ ions. As a result, cobalamin complexes with all NO, H₂O, O₂ and NO₂⁻ may be formed in the course of the cobalamin complexation process. Their co-existence depends on a respective complex formation constant and, consequently, equilibrium constants of ligand exchange reactions as well as the ratio of kinetic constants of the above-mentioned processes. Therefore, not only the exact values of parameters such as binding energies are important, but also the relations between them.

The aim of the present work is to investigate complexation properties of small, inorganic, biologically relevant species of AB and AB₂ type, namely NO, O₂, H₂O, and NO₂⁻, towards cobalamins in which cobalt ion is on both +2 and +3 oxidation states. The possibility for the existence of six- and five-coordinate (“base-off”) cobalamin complexes is discussed. Five-coordinate complexes might be created as cobalamin complexes formed at low pH. In the latter case, the nitrogen atoms from dimethylbenzimidazole become protonated leading to the dissociation of the cobalt–dimethylbenzimidazole bond. The studies on the interactions of all above-mentioned ligands with five-coordinate imidazole–cobalamins and four-coordinate cobalamins would allow one to get some knowledge about the equilibria of different possibly existing complexes in water solution in aerobic conditions.

2 Methodology

To study ligand binding properties of cobalamin complexes the density functional theory (DFT) was applied as implemented in StoBe program [23]. The selection of the methodology follows a fact that at present it is one of the most powerful techniques of quantum chemistry frequently used to study biological and coordination systems. The DFT approach combines high accuracy with relatively low cost of calculations. In the present studies Kohn–Sham molecular orbitals were represented by linear combinations of extended all-electron DZVP quality basis sets of Gauss type atomic

orbitals [24,25]. The studied complexes were characterized by geometry parameters such as bond lengths and valence angles resulting from geometry optimization procedure performed within the LDA-VWN functional [26]. There is no doubt that LDA-VWN functional performs poorer than other GGA functionals in geometry optimization (e.g. bond distances are shorter), but the calculations within LDA scheme are much faster than with the use of GGA-class functional. However, we would like to point out that the present analysis is aimed to compare the studied systems rather than to get the absolute values. Moreover, it is found that the geometry predictions within LDA theory are usually quite reasonable due to a fact that a number of localized electron pairs is constant during stretching and bending of bonds near an equilibrium geometry. This results in cancellation of systematic error of coulomb correlation energy in comparable geometries of a molecule. The electronic (Mayer bond orders [27]) and energetic (ligand binding energies) parameters of the systems were obtained at the GGA-RPBE level [28,29]. The latter were calculated as the difference between the energy of the complex and the sum of the energies of the isolated fragments. The chosen approach has already been proved to accurately reproduce ligand binding energies to cobalamins as well as the structural parameters that fit well the experimental data [22].

It is important to mention that computed in that way ligand binding energies are only internal energies and do not contain any thermal corrections or zero-point vibrational energies (ZPVE). Up to our knowledge, there are only three papers discussing the ZPVE corrections for ligand dissociation energies in cobalamin complexes. Despite the presented discrepancies in ZPVE values, one can see that this correction is comparable within the whole set of compounds and within a methodology used for calculations. One may thus postulate the same behavior of ZPVE for binding of inorganic ligands to cobalamins. Of course, this assumption should be taken with caution. Nevertheless, if ZPVE values are systematic, they should not count much while comparing binding of different ligands to the same cobalamin complex or comparing binding of the same ligand to different forms of cobalamins.

The whole five-coordinate cobalamin complex contains 179 atoms, which is too much to treat with such an accurate method. Therefore, the reduction of the system was necessary and the model for theoretical treatment that includes cobalt ion, corrin ring (Cor) and imidazole ligand (Im) as shown in Fig. 1 was chosen keeping in mind the exact description of the first coordination sphere of the cobalt ion. In such a model the first coordination sphere of the cobalt ion is retained. In order to obtain the stable structure of the corrin ring, full geometry optimization was done for [Co^{II}Cor]⁺ (formally containing cobalt(II) ion) and [Co^{III}Cor]²⁺ (formally containing cobalt(III) ion). While doing this we were aware of the fact that, up to our knowledge, there are no experimental

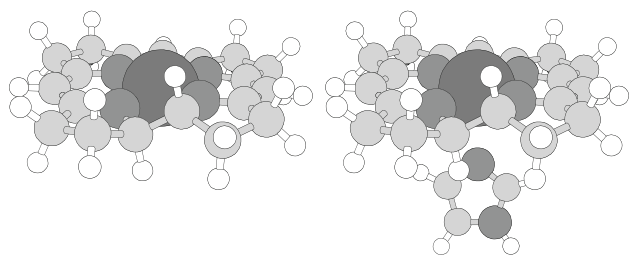


Fig. 1 Model of four- and five-coordinate cobalamins selected for theoretical investigation

evidences for the presence of four-coordinate cobalamins with cobalt on either +2 or +3 oxidation state. However, we performed geometry optimization for these non-existing species in order to be able to compute ligand binding energies as an energy difference between a complex and a sum of its fragments. The resulting geometry of the macrocyclic ligand was then used as fixed in the subsequent calculations of the studied five- and six-coordinate complexes. For the latter, axial ligands and the central atom were allowed to move. This simplification speeds up calculations and may be justified as follows. In paper by Selcuki et al. [21] different theoretical methods were used to fully optimize different cobalamin systems with and without axial ligands. It was observed that the geometry (so-called “folding angle”) of the corrin ring is invariable upon binding of NO and H₂O to both cob(II)- and cob(III)alamins. Moreover, in the paper reporting crystal structure of the dioxygen derivative of cob(II)alamin [31], there was no difference in “folding angle” of the corrin ligand with respect to the crystal structure of free cob(II)alamin. All systems were calculated as low spin.

3 Results and discussion

The discussion of the geometry parameters as well as ligand binding energies of the investigated complexes is divided into four subsections, each devoted to the complexes formed

with one of the studied inorganic ligands: NO, O₂, NO₂[−], and H₂O. The description of the systems within each group of compounds is organized in such a way that both five coordinate cob(II)alamin and cob(III)alamin complexes are discussed first, followed by the comparison of the parameters of six-coordinate cob(II)alamin and cob(III)alamin complexes with imidazole as the sixth ligand. Obtained results are compared with experimental and/or other computational data if these are available.

3.1 Nitrosyl complexes

The calculated parameters of all the studied nitrosyl complexes, namely [Co^{II}CorNO]⁺, [Co^{III}CorNO]²⁺, [Co^{II}CorImNO]⁺, and [Co^{III}CorImNO]²⁺, are gathered in Table 1.

As far as both five-coordinate systems are concerned, one sees that nitric oxide forms short and covalent bonds with cobalt on both +2 and +3 oxidation states. The Co–NO bond is shorter and more covalent in nitrosyl-cob(II)alamin than in nitrosyl-cob(III)alamin. In both systems the NO binding to cobalamin complexes is favorable from the thermodynamic point of view. Binding to cob(II)alamin is preferred by 24 kJ mol^{−1} over binding to cob(III)alamin. The length of bonded NO is practically the same in both the investigated complexes as in free NO species and the only perturbation induced by the coordination to the metal center is the lowering of N–O bond order in the resulting five-coordinate complexes.

Although, up to our knowledge, the X-ray structures of any of six-coordinate nitrosyl systems are not known, one can find some indications regarding the structure of studied [Co^{II}CorImNO]⁺ system. The strong bending of the Co–N–O group (117°) corroborates well with experimental results of both resonance Raman spectroscopy [17] and ¹⁵N NMR [15] investigations which indicate that in the resulting structure the nitrosyl ligand is strongly bent.

Table 1 Geometric and electronic parameters of nitrite-cobalamins

System	[CoCorNO] ⁺	[CoCorNO] ²⁺	[CoCorImNO] ⁺	[CoCorImNO] ²⁺
E_b^{NO}	−110	−86	−75, −76	−3
E_b^{Im}	–	–	3 (−33)	−100 (−184)
Geometry				
Co–NO	179	182	184 [185–192]	191 [186–335]
N–O	118 (117)	117 (117)	119 (117) [118–119]	116 (117) [112–117]
Co–Im	–	–	218 (202) [210–223]	195 (184) [193–200]
Co–N–O	119°	122°	117° [118° – 121°]	124° [117° – 130°]
Bond orders				
Co–NO	1.22	1.13	1.10	0.82
N–O	1.77 (2.22)	1.86 (2.22)	1.78 (2.22)	1.96 (2.22)
Co–Im	–	–	0.40 (0.57)	0.70 (0.82)

Distances are in pm, energies in kJ mol^{−1}. In parenthesis, the values for isolated molecules are given as such isolated NO, [CoCorIm]⁺ and [CoCorIm]²⁺ are taken. In italics, the experimental values are listed [16]. In square brackets, other theoretical results are listed [21]

Table 2 The comparison of NO and H₂O binding energies [kJ mol⁻¹] for six-coordinate cobalamin complexes obtained in this paper and by different methods in Ref. [21]

System	[CoCorImNO] ⁺	[CoCorImNO] ²⁺	[CoCorImH ₂ O] ⁺	[CoCorImH ₂ O] ²⁺
This paper	-75	-3	10	-16
B3LYP	-14	-37	-21	-82
PW91	-121	-100	-29	-100
HF	279	-8	-16	-96
MP2	-644	-26	-623	-139
ROMP2	-14	85	-14	-
Exp [15]	-76 ^a	-	-	-

^a pH = 7.4

The comparison of the parameters describing both six-coordinate complexes reveals that in analogy to five-coordinate complexes, NO would bound to cob(II)alamin complex rather than cob(III)alamin (difference of 72 kJ mol⁻¹ in NO binding energies in favor for cob(II)alamin). This finding is in good agreement with experimental data [16] indicating cob(II)alamin as a main target for NO. In fact, there are also some experimental evidence that NO may be also bound by cob(III)alamins [18], but these results were obtained with glutathionyl derivative of cob(III)alamin indicating that in that case NO reacts with sulfur atom from SH group, forming nitrosothiols, rather than directly with cobalt(III) ion.

While comparing obtained structural parameters for both six-coordinate systems with those already reported by Selcuki et al. [21], one immediately sees the similarities. In [Co^{II}CorImNO]⁺ the lengths of the Co–Im (218 pm) and N–O (119 pm) bonds are both within the range of these listed in the cited paper (210–223 and 118–119 pm, respectively). The Co–NO distance obtained in the present studies (184 pm) is slightly shorter as compared to 185–192 pm published in [21]. Similarly, the Co–N–O titling angle (117°) is a little underestimated with respect to the range of 118–121° taken from [21]. In [Co^{III}CorImNO]²⁺ the lengths of the discussed bonds (Co–NO = 191 pm, N–O = 116 pm, Co–Im 195 pm) fall into the ranges of those calculated by different approaches by Selcuki et al. [21] (186–335, 112–117 pm, and 193–200 pm, respectively). As far as NO binding energies are concerned, values obtained in the present approach give better agreement with the experiment. Whereas each of the methods employed in [21] to calculate NO binding energy failed to reproduce the experimental value of -76 kJ mol⁻¹ (see Table 2), our method yields -75 kJ mol⁻¹ that well fits the experiment. One should notice that experiment measures the energy that should be formally defined as energy of the bond, in other words, the energy needed to split a given bond. Therefore, the values of both experimental and theoretical energies have the same value, but the opposite signs. In the present paper, the signs of experimental binding energies are inverted in order to facilitate the discussion. As there is no experimental data regarding NO binding to cob(III)alamin, the valuation of our as well as the other calculations cannot be

done. One should only point out that NO binding energy computed here being equal to -3 kJ mol⁻¹ is smaller than all but one reported in [21] (-8 kJ mol⁻¹ for HF, -26 kJ mol⁻¹ for MP2, +85 kJ mol⁻¹ for ROMP2, -37 kJ mol⁻¹ for B3LYP, and -100 kJ mol⁻¹ for PW91) suggesting weaker bonding of NO in [Co^{III}CorImNO]²⁺.

Thermodynamics of the studied complexes reveals that the formation of six-coordinate nitrosyl-cob(II)alamin complex might be followed by the dissociation of the imidazole ligand. The statement is supported by the observation that the coordination of NO species to five-coordinate cob(II)alamin ([Co^{II}CorIm]⁺) results in elongation of Co–Im bond by 16 pm as compared to the respective value in [Co^{II}CorIm]⁺, yielding the Co–Im bond of 218 pm (NO-binding induced *trans influence*). The increase of the Co–Im distance is accompanied by lowering of this bond order from 0.57 in [Co^{II}CorIm]⁺ to 0.40 in the resulting structure. Moreover, imidazole binding energy in the studied system is very small and positive (3 kJ mol⁻¹) which suggests that the Co–Im bond is thermodynamically unstable. As a result, five-coordinate nitrosyl complex would be formed. It should be stressed, however, that in complex with cob(III)alamin, the Co–Im bond is thermodynamically stable and no imidazole dissociation should be expected. What is more, in case of cob(III)alamin solution, five-coordinate imidazole-cob(III)alamin form will dominate over six-coordinate nitrosyl one.

Finally, the comparison of the Co–NO bond parameters such as length, bond order and NO binding energy in both six-coordinate and five-coordinate complexes demonstrates that the coordination of the imidazole ligand weakens the investigated bond. Similar mutual dependence of trans bonds strength was already found for NO binding to ferrous heme iron ([30] and references therein). In those systems NO binding dissociates trans ligand, and binding of trans ligand to the five-coordinate nitrosyl complex enhances the dissociation of the NO ligand.

3.2 Dioxygen complexes

The calculated parameters of the investigated dioxygen adducts that are [Co^{II}CorO₂]⁺, [Co^{III}CorO₂]²⁺, [Co^{II}CorImO₂]⁺ and [Co^{III}CorImO₂]²⁺ are listed in Table 3.

Table 3 Geometric and electronic parameters of O₂–cobalamins

System	[CoCorO ₂] ⁺	[CoCorO ₂] ²⁺	[CoCorImO ₂] ⁺	[CoCorImO ₂] ²⁺
$E_b^{O_2}$	–29	–1	–26, –34, –31, –25	95
E_b^{Im}	–	–	–30 (–33)	–87 (–184)
Geometry				
Co–O ₂	183	176	191, 193	188
O–O	125 (122)	127 (122)	127 (122) 132	127 (122)
Co–Im	–	–	199 (202) 206	201 (184)
Co–O–O	116°	117°	116°, 120.0°	115°
Bond orders				
Co–O ₂	0.83	0.93	0.74	0.78
O–O	1.39 (1.87)	1.31 (1.87)	1.42 (1.87)	1.37 (1.87)
Co–Im	–	–	0.59 (0.57)	0.69 (0.82)

Distances are in pm, energies in kJ mol^{–1}. In parenthesis, the values for isolated molecules are given - as such isolated O₂, [CoCorIm]⁺ and [CoCorIm]²⁺ are taken. In italics, the experimental values are listed [31–33]

The major difference between five-coordinate complexes is the O₂ binding energy (–29 kJ mol^{–1} in [Co^{II}CorO₂]⁺ vs. –1 kJ mol^{–1} in [Co^{III}CorO₂]²⁺). One immediately notices that the O₂ ligand prefers binding to cob(II)alamin than cob(III)alamin. While the binding of O₂ to form [Co^{II}CorO₂]⁺ is thermodynamically privileged, the formation of the analogous bond in [Co^{III}CorO₂]²⁺ is thermodynamically neutral. The geometry of O₂ ligand is similar in both studied five-coordinate complexes. In both systems the O–O distance in bound O₂ ligand is expanded and, as a result, the O–O bond order is lowered. The tilting angle in both complexes is almost the same. The O–O bond is longer and less covalent in [Co^{II}CorO₂]⁺ than in [Co^{III}CorO₂]²⁺.

The same as in already discussed five-coordinate oxygen adducts, O₂ binding energy is the parameter that most differentiates the investigated structures. Binding of this ligand to cob(III)alamin is strongly unfavorable ($E_b^{O_2} = 95$ kJ mol^{–1}) in contrast to cob(II)alamin case, where the O₂ molecule forms weak, but thermodynamically stable bond with cobalt (II) ion ($E_b^{O_2} = -26$ kJ mol^{–1}). Other parameters of the studied structures are comparable and no major differences are found between parameters of the O–O bond in both cob(II)alamin and cob(III)alamin complexes. As the Co–Im bond parameters are concerned, one should notice that in both six-coordinate compounds the Co–Im distance is very similar (199 pm in [Co^{II}CorImO₂]⁺ vs. 201 pm in [Co^{III}CorImO₂]²⁺). The Co–Im bond order is lower by 0.10 in [Co^{II}CorImO₂]⁺ in comparison to [Co^{III}CorImO₂]²⁺. Consequently, imidazole is bound weaker by 57 kJ mol^{–1} in the cob(II)alamin complex than in the cob(III)alamin one. Nevertheless, in both complexes the Co–Im bond is thermodynamically stable.

The crystallographic data for six-coordinate O₂–cob(II)alamin adduct are published in Ref. [31]. The calculated oxygen-metal distance agrees well with the measured value (191 vs. 194 pm, respectively). The theoretical O₂ binding energy (–26 kJ mol^{–1}) fits well the experimental findings

(–25 to –34 kJ mol^{–1}) [31–33]. The O–O distance in coordinated ligand is very close to 132 pm derived from experiment [31]. The calculated Co–O–O angle is smaller only by 4° than obtained from X-ray data and amounts to 116°. The Co–Im bond length is calculated to be 199 pm that is very close to the experimental value of 206 pm [31].

3.3 Nitrite complexes

Table 4 summarizes selected structural parameters for the studied nitrite cobalamins that are [Co^{II}CorNO₂], [Co^{III}CorNO₂]⁺, [Co^{II}CorImNO₂], and [Co^{III}CorImNO₂]⁺ complexes.

The comparison of both five-coordinate complexes reveals that NO₂[–] forms shorter and more covalent bond with cobalt (III) than with cobalt(II) ion. The formation of both complexes is strongly favored by very high binding energies of nitrite to cobalamins. Binding by cob(III)alamin is thermodynamically more preferred than binding by cob(II)alamin. The formation of both five-coordinate complexes, i.e., with [Co^{II}Cor]⁺ and [Co^{III}Cor]²⁺ implies only slight shortening (by 4–6 pm) of the N–O bonds in the investigated ligand accompanied by only slight changes in their bond orders.

As can be seen from Table 4, the lengths and bond orders of bonds formed by cobalt center with NO₂[–] and Im axial ligands are almost the same in both six-coordinate complexes. The structural data for six-coordinate nitrite cob(III)alamin complex are available [34]. The calculated Co–NO₂[–] distance (194 pm) is in a good agreement with experimental value of 194.1 pm. The same, obtained length of the Co–Im bond is equal to 198 pm, that is smaller only by 3 pm than the value derived from experiment (200.8) [34]. Interestingly, the imidazole binding energy is the parameter that differentiates both six-coordinate systems. The Co–Im bond is stable in terms of thermodynamics in [Co^{III}CorImNO₂]⁺, whereas it is very unstable in [Co^{II}CorImNO₂] system—the imidazole binding energy has relatively high, positive

Table 4 Geometric and electronic parameters of NO_2^- -cobalamins

System	[CoCorNO ₂]	[CoCorNO ₂] ⁺	[CoCorImNO ₂]	[CoCorImNO ₂] ⁺
$E_b^{\text{NO}_2}$	-410	-914	-310	-754
BP86	-	-	-330*	-783*
Solv corr.	-	-	-120*	46*
E_b^{Im}	-	-	67 (-33)	-21 (-184)
Geometry				
Co-NO ₂ ⁻	203	184	190	191, <i>194.1</i>
N-O	124/124 (128/128)	122/122 (128/128)	124/123 (128/128)	123/123 (128/128)
Co-Im	-	-	197 (202)	198 (184) <i>200.8</i>
Bond orders				
Co-NO ₂ ⁻	0.62	0.78	0.69	0.68
N-O	1.58/1.54 (1.55/1.55)	1.56/1.56 (1.55/1.55)	1.53/1.57 (1.55/1.55)	1.55/1.57 (1.55/1.55)
Co-Im	-	-	0.57 (0.57)	0.58 (0.82)

Distances are in pm, energies in kJ mol^{-1} . In parenthesis, the values for isolated molecules are given – as such isolated NO_2^- , $[\text{CoCorIm}]^+$ and $[\text{CoCorIm}]^{2+}$ are taken. In italics, the experimental values are listed [34]. The values marked with asterisk refer to binding energies calculated here with BP86 functional (the first row) and further corrected for solvent within simple continuum model (the second row)

value (67 kJ mol^{-1}). This finding may suggest that the six-coordinate nitrite-cob(II)alamin complex would convert into five-coordinate nitrite-cob(II)alamin complex in real system. What is more, the nitrite complexes are more likely to occur at low pH, when cobalamin complexes may be in “base-off” form which means that cobalt ion is not coordinated by the axial dimethylbenzimidazole ligand (modeled here by imidazole).

Similar to what was found for five-coordinate nitrite complexes, the NO_2^- ion is strongly bound in both six-coordinate complexes and binding to cob(III)alamin is preferred over binding to cob(II)alamin (difference in binding energies amounts to 444 kJ mol^{-1}).

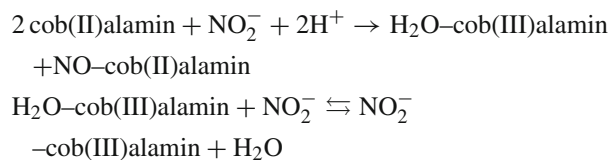
As stated before, NO_2^- binding energies are very high for all studied systems. One should point out, however, that such high binding energies may partially result from the fact that nitrite adduct formation implies partial (in case of cob(III)alamin complexes) or total (in case of cob(II)alamin complexes) charge compensation. Such a reaction is privileged in gaseous phase for which calculations are done. Therefore, when one wants to consider reactions in water solution, it is necessary to be aware of the fact that all diluted species are hydrated. According to Born theory [35] the solvation energy is proportional to square of the charge of an ion (z_i) divided by its radius (r_i):

$$E_{\text{solv}} \sim -z_i^2/r_i \quad (1)$$

Assuming that once a small ligand is bound to cobalamin, the radius of the whole adduct is increasing insignificantly, the charge of the ion stays the only variable in Eq. 1. While the reaction comprises binding of the neutral ligand, one may assume that the solvation energy is constant. The situation

is different when nitrite reacts with cobalamin system. Therefore, in these systems we decided to correct the obtained binding energies by the solvation energy. The latter were obtained using COSMO model within BP86 as implemented in Turbomole 5.7 [36]. The change of methodology follows from the fact that it is not possible to account for solvent effects in StoBe program. The results of our calculations are included in Table 4. Firstly, one immediately sees that the binding energies calculated within BP86 without solvent correction are of the same order as the results of VWN/RPBE ones. For nitrite binding to cob(II)alamin this is -330 against -310 kJ mol^{-1} within RPBE, and for nitrite binding to cob(III)alamin equals to -783 against -754 kJ mol^{-1} within RPBE. Secondly, when the solvent is taken into account, one see the change in the complexes behavior. The nitrite binds only to cob(III)alamin (binding energy amounts to -120 kJ mol^{-1}), whereas binding to cob(II)alamin is thermodynamically not privileged (binding energy is positive and equals to 46 kJ mol^{-1}).

There are some reports in the literature on the experiments with nitrite ions with cob(II)alamin in O_2 -free water solution [16]. This reaction was found to yield mainly NO_2^- -cob(III)alamin adduct as a result of the following reactions:



where nitrite-cob(II)alamin complex is rarely detected. This is attributed to the fact that the formation of nitrite-cob(II)alamin complex is several order of magnitude slower than that of nitrosyl-cob(II)alamin one.

Table 5 Geometric and electronic parameters of H₂O–cobalamins

System	[CoCorH ₂ O] ⁺	[CoCorH ₂ O] ²⁺	[CoCorImH ₂ O] ⁺	[CoCorImH ₂ O] ²⁺
$E_b^{\text{H}_2\text{O}}$	–11	–65	10	–16
E_b^{Im}	–	–	–13 (–33)	–134 (–184)
Geometry				
Co–H ₂ O	214	190	253 [333–347]	200 [198–204] <i>195.2</i>
O–H	98/98 (98/98)	0.99/0.99 (98/98)	98/98 (98/98)	99/99 (98/98)
Co–Im	–	–	204 (202) [209–230]	187 (184) [188–209] <i>192.5</i>
Bond orders				
Co–H ₂ O	0.32	0.50	0.15	0.37
O–H	0.75/0.75 (0.81/0.81)	0.72/0.72 (0.81/0.81)	0.77/0.78 (0.81/0.81)	0.74/0.75 (0.81/0.81)
Co–Im	–	–	0.53 (0.57)	0.77 (0.82)

Distances are in pm, energies in kJ mol^{–1}. In parenthesis, the values for isolated molecules are given – as such isolated H₂O, [CoCorIm]⁺ and [CoCorIm]²⁺ are taken. In italics, the experimental values are given [37]. In square brackets, other theoretical results are listed [21]

3.4 Aqua complexes

The calculated parameters of all studied aqua complexes that are both five-coordinate [Co^{II}CorH₂O]⁺, [Co^{III}CorH₂O]²⁺ and six-coordinate [Co^{II}CorImH₂O]⁺, [Co^{III}CorImH₂O]²⁺ species are listed in Table 5.

There is a significant difference in parameters describing Co–H₂O bond in both five-coordinate complexes. The Co(II)–H₂O bond is longer by 24 pm and has smaller bond order by 0.28 than the Co(III)–H₂O one. Consequently, H₂O binding is preferred by 54 kJ mol^{–1} to cob(III)alamin than cob(II)alamin. The O–H bond lengths of H₂O ligand are practically intact in both five-coordinate complexes as compared to the O–H bonds in isolated H₂O molecule, while bond orders are diminished in both five-coordinate aqua cob(II)alamin and cob(III)alamin systems. The weakening of the O–H bonds is slightly more pronounced in [Co^{III}CorH₂O]²⁺ than in [Co^{II}CorH₂O]⁺.

H₂O with cob(III)alamin forms shorter bond by 53 pm with a higher bond order by 0.22 than water with cob(II)alamin. Moreover, the Co–H₂O bond is stabilized by 26 kJ mol^{–1} more in [Co^{III}CorImH₂O]²⁺ than in [Co^{II}CorImH₂O]⁺. Therefore, the obtained results allow one to conclude that the water ligand preferentially coordinates cob(III)alamins not cob(II)alamins. These findings are in agreement with experimental results. The H₂O–cob(III)alamin, so-called aquacobalamin, is widely known as vitamin B_{12a}. Crystallographic data for [Co^{III}CorImH₂O]²⁺ complex are thus known and published in Ref. [37]. Our calculations indicate that the water ligand is bound at a distance of 200 pm from the cobalt (III) center which is in good agreement with the experimentally measured value of 195.2 pm. The imidazole ligand is coordinated at the distance of 187 pm from the metal center, which agrees well with 192.5 pm determined by experiment.

According to [16], aqua-complex with cob(II)alamin is formed as a transient species after a photolysis of nitrosyl-

cob(II)alamin complex due to the excess water in proximity to the ligand binding site.

The results of calculations for both six-coordinate systems are already published in [21] allowing for the comparison with these ones reported here. Even though the length of the Co–Im bond (204 pm) in [Co^{II}CorImH₂O]⁺ is not far from 209–230 pm published in [21], there is a strong disagreement regarding the Co–H₂O distance (253 pm in this paper vs. 333–347 pm in [21]). The fact that LDA is known to often underestimate bond lengths may explain this discrepancy. Surprisingly, H₂O binding energy calculated in this study (10 kJ mol^{–1}) suggesting thermodynamically unstable bond, is in contrast to the values reported in [21], where Co–H₂O is postulated to be weak, but thermodynamically stable (refer to Table 2 for comparison). The MP2 calculations, which indicates very stable bond ($E_b^{\text{H}_2\text{O}} = -623$ kJ mol^{–1}), is the only exception. There are no data regarding O–H bond lengths in [21]. Further, as far as [Co^{III}CorImH₂O]²⁺ is concerned, one can see that there is an agreement regarding investigated bond lengths, i.e., 200 vs. 198–204 pm for Co–H₂O and 187 vs. 188–209 pm for Co–Im. The H₂O binding energy obtained in the present calculations (–16 kJ mol^{–1}) is lower than all reported in [21] (see Table 2), as the methods employed there give the values ranging from –82 kJ mol^{–1} for B3LYP to –139 kJ mol^{–1} for MP2. The same as for [Co^{II}CorImH₂O]⁺, the O–H bond lengths are not listed in [21].

4 Conclusions

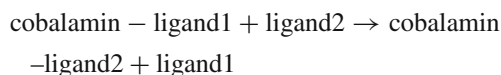
In summary, the present study provides theoretical description of five- and six-coordinate forms of both cob(II)- and cob(III)alamin complexes with selected, inorganic ligands, namely NO, O₂, NO₂[–] and H₂O. Our interest in these ligands is mainly attributed to their significance in biochemistry including interactions with cobalamins. In a water solution

in aerobic conditions NO competes with other ligands such as O₂, NO₂⁻ and H₂O for the same binding site in a metal complex. Thus, the importance of such a comparative study of the possible adducts seems to be unquestionable.

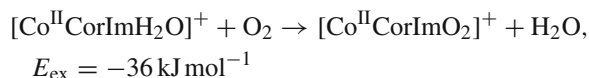
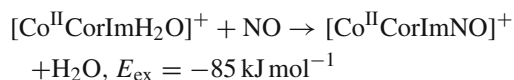
The results obtained for all cobalamin complexes with small, inorganic ligands show that NO ligand forms the shortest and the most covalent bonds with cobalt complexes on +2 oxidation state. In case of Co(III) complexes, the shortest Co–ligand bond is formed with the O₂ molecule, but still nitric oxide forms the most covalent bonds.

Furthermore, the analysis of all studied systems reveals that the coordination of both AB (NO and O₂) and AB₂ (NO₂⁻ and H₂O) type of small ligands to cobalt ion results either in none or only in minor perturbations in the A–B distances in each of the considered species (the largest change of 6 pm is found for NO₂⁻ ligand in [Co^{III}CorImNO₂]⁺ complex). However, the changes in the A–B bond orders induced by the coordination to metal ion allow one to divide the studied small ligands into two groups: the first one includes the AB type molecules (NO and O₂) whereas the second the AB₂ type species (H₂O and NO₂⁻). The A–B bond order is strongly diminished in ligands from the first group (for NO the changes in the N–O bond orders range from 0.26 in [Co^{III}CorImNO]²⁺ to 0.45 in [Co^{II}CorImNO]⁺, and for O₂ the O–O bond orders vary from 0.45 in [Co^{II}CorImO₂]⁺ to 0.56 in [Co^{III}CorImO₂]²⁺). In contrast, the A–B bonds in ligands from the second group are not or almost not affected by the coordination. For the H₂O ligand the largest change in the O–H bond order is found to be 0.09 in [Co^{III}CorH₂O]²⁺, whereas for the NO₂⁻ ion the N–O bond order value varies from +0.03 in [Co^{II}CorNO₂] to –0.02 in both [Co^{II}CorImNO₂] and [Co^{III}CorImNO₂]⁺.

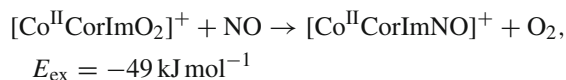
The comparison of small ligand binding energies may also give some insights into the equilibrium between different species in water solution of cobalamins under the aerobic conditions. This may be done if one compares the energies of the ligand exchange reaction (E_{ex}):



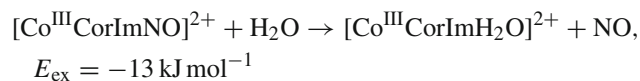
In cob(II)alamin solution, both O₂ and NO ligands would replace H₂O bonded to cob(II)alamin:



Nitric oxide would also react with the oxygen derivative of cob(II)alamin according to the reaction:



In cob(III)alamin solution, by contrast, H₂O would exchange bound NO:



It should be pointed out, however, that the ligand exchange energy is quite small for the investigated reaction. Therefore, one should be aware that in real systems there are a number of factors like temperature or the effect of concentration that may affect the equilibrium between the two forms.

Presented results regarding equilibria between different forms of NO and H₂O cob(II)alamin and cob(III)alamin complexes support the hypothesis that cob(II)alamin favors NO, whereas cob(III)alamin favors H₂O [16]. [Co^{III}CorImO₂]²⁺ complex will not be formed. NO₂⁻ ion would react with all cob(III)alamin species present in solution, which is reflected by the UV/V spectroscopy observations of [Co^{III}CorImNO₂]⁺ formation [16].

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