

NO Binding to Cobalamin: Influence of the Metal Oxidation State

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Density functional and molecular orbital theory calculations on models for cobalamin suggest that NO binds similarly to the Co(II) and Co(III) oxidation states. However, Co(III) can bind water far more strongly than Co(II) as a sixth ligand, so that the competition between water and NO complexation strongly favors water for Co(III) in the gas phase. Although the Co(II) oxidation state is found to bind water slightly more strongly than NO in the gas phase, the inclusion of solvation effects via the polarizable continuum model makes NO binding more favorable. Thus, the experimentally observed ability of cob(II)alamin to bind NO in aqueous solution is the result of its weak complexation with water and the relatively poor solvation of NO. Calculated vibrational frequencies support the interpretation of the cob(II)alamin–NO complex as being cob(III)alamin–NO[−], although the DFT calculations underestimate the degree of charge transfer in comparison to Hartree–Fock calculations.

Introduction

The many studies on the structure and reactivity of vitamin B₁₂ have resulted in much interest in the mechanisms of its biological functions. One of the most recent topics of interest concerns the role of aquacobalamin (vitamin B_{12a}, Cbl(III)–H₂O) and reduced cobalamin (vitamin B_{12r}, Cbl(II); Chart 1) in modifying the physiological action of nitric oxide in vivo. Such interactions have been suggested to play an important role in various biological processes involving nitric oxide.^{1,2} A possible explanation for these observations involved the effective formation of a nitrosyl complex between aquacobalamin and NO, according to the overall reaction given in (1), by which aquacobalamin acts as a biological scavenger of NO.^{1,2}



Detailed spectroscopic and kinetic studies^{1b} have shown that no direct interaction between NO and aquacobalamin

occurs.^{1b} Reaction 1, therefore, cannot account for the observed physiological effects. However, UV–vis, EPR, and resonance Raman spectroscopic studies suggest that the reduced form of vitamin B₁₂ reacts with nitric oxide to form a Cbl(II)–NO complex^{2,3} with a formation constant of $(1.0 \pm 0.5) \times 10^8 \text{ M}^{-1}$. A recent detailed spectroscopic and kinetic investigation of this reaction⁴ indicated that reduced cobalamin binds NO extremely rapidly and efficiently and that the formal character of the nitrosyl adduct is Co^{III}–NO[−], as suggested by ¹⁵N NMR measurements.⁴ Thus, the effective binding of NO to reduced cobalamin (which is present under physiological conditions^{5–7}) may be responsible for inhibition of NO actions in vivo, as well as NO-induced inactivation of selected cobalamin-dependent enzymes.⁸ Furthermore, such reactions also seem to play an important role in the interaction of reduced cobalamin and nitroprusside, a well-recognized hypotensive agent.⁹

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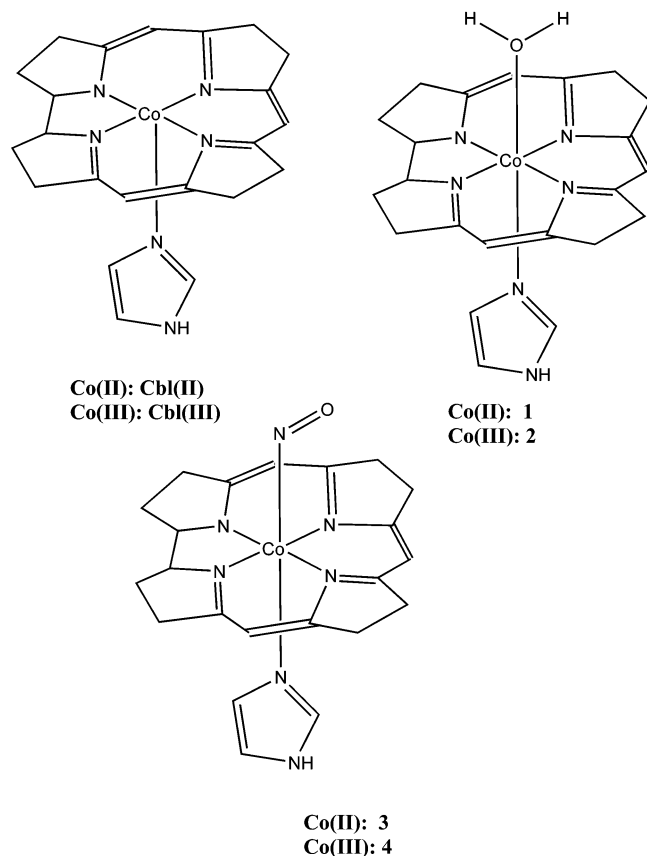
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Chart 1



There have been many theoretical studies of cobalamins, almost all of which used density functional theory (DFT). Kozłowski¹⁰ has reviewed the work published before 2001, and more recently Maseras and co-workers^{11,12} have also reported DFT studies on cobalamin models for B₁₂ cofactors. These studies have concentrated on the strength and mechanism of cleavage of the Co–C bond and on the effect of trans-ligands on these properties. Nevertheless, the authors have drawn some conclusions that are useful in judging the reliability of different calculational levels. There are some indications¹³ that the Becke 3-parameter hybrid functional¹⁴ with the Lee–Yang–Parr correlation functional¹⁵ (B3LYP)¹⁶ underestimates the Co–C bond dissociation energy by about 10 kcal mol⁻¹ and that the scaling factor required for the Co–C stretching vibration is unusually small.¹⁷ Despite these potential problems, the B3LYP functional has been used for the vast majority, if not all, of the DFT studies reported so far for cobalamins.^{10–13,17}

We now report a theoretical study as to why NO prefers to bind to Co^{II} and not Co^{III} in cobalamin. Because of the

uncertainty as to the suitability of the B3LYP functional, we have also used the Perdew–Wang 91 combination of exchange and correlation functionals (PW91),¹⁸ as well as Hartree–Fock optimizations and single-point calculations using a second-order Møller–Plesset (MP2) correction for electron correlation (MP2)¹⁹ to assess the applicability of different techniques and levels of theory. Our results provide new insight into cobalamin–NO binding in both oxidation states. This study is part of a wider analysis of the redox-controlled binding of NO by transition metal complexes.^{20–24} Rather than to use the entire cobalamin, we have used the model compounds Cbl(II) and Cbl(III), their water complexes **1** and **2**, respectively, and their NO complexes **3** and **4**, respectively.

Methods

All structures were initially optimized at the PW91/DNP level using the DMOL³ program²⁵ in Materials Studio 2.2.²⁶ These geometries were used as starting points for HF/6-31G(d), PW91/6-31G(d), and B3LYP/6-31G(d) geometry optimizations using the Gaussian 98²⁷ program package. All structures were characterized as true minima by calculating their normal vibrations within the harmonic approximation. Single-point self-consistent reaction field (SCRF)²⁸ calculations were used to calculate the solvation energies in aqueous solution. The PCM model as implemented in Gaussian 98²⁹ was used for solvent calculations. The following discussion will be based on PW91/6-31G(d)//PW91/6-31G(d) results with corrections for zero-point vibrational energies unless otherwise stated.

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Table 1. Calculated Total Energies (E_{TOT} , in hartrees), Zero-Point Energies (ZPE, in kcal mol⁻¹), Total Energies in Solution (E_{SOLV} , in hartrees), Binding Energies (E_{BIND} , in kcal mol⁻¹), Exchange Energies ($E_{exchange}$ in kcal mol⁻¹), and Ionization Potentials (IP, eV) for Cobalamins and Their Complexes at the B3LYP/6-31G* Level

	E_{TOT}	ZPE	E_{BIND}^a (vacuum)	$E_{exchange}^b$ (vacuum)	IP (vacuum)	E_{SOLV}	IP (Water)	$E_{exchange}^b$ (water)
B3LYP/6-31G(d)								
Cbl(II)	-2564.503 91	282.8			8.87	-2564.556 50	7.48	
Cbl(III)	-2564.177 88	284.2				-2564.281 59		
Cbl(II)-H ₂ O, 1	-2640.922 41	297.2	-4.9		8.17	-2640.970 30	3.78	
Cbl(III)-H ₂ O, 2	-2640.622 25	300.2	-19.5			-2640.831 55		
Cbl(II)-NO, 3	-2694.400 82	287.8	-3.3	+1.6	8.68	-2694.442 04	4.49	-1.5
Cbl(III)-NO, 4	-2694.081 95	288.1	-8.9	+11.4		-2694.277 00		+12.5
H ₂ O	-76.408 93	13.3				-76.418 30		
NO	-129.888 16	2.8				-129.885 86		
PW91/6-31G(d)								
Cbl(II)	-2564.190 10	276.0			8.60	-2564.244 08	4.19	
Cbl(III)	-2563.875 69	277.0				-2564.091 72		
Cbl(II)-H ₂ O, 1	-2640.582 13	289.9	-6.9			-2640.65247	4.19	
Cbl(III)-H ₂ O, 2	-2640.293 23	292.8	-23.8		7.99	-2640.502 99		
Cbl(II)-NO, 3	-2694.091 79	280.6	-28.8	-21.9		-2694.134 99	4.75	-12.1
Cbl(III)-NO, 4	-2693.768 38	280.9	-23.9	-0.1	8.81	-2693.960 35		+4.0
H ₂ O	-76.37962	13.0				-76.388 77		
NO	-129.852 73	2.7				-129.850 40		
HF/6-31G(d)								
Cbl(II)	-2555.507 89	302.6			9.54	-2555.561 21	5.12	
Cbl(III)	-2555.161 28	305.1				-2555.376 94		
Cbl(II)-H ₂ O, 1	-2631.526 23	318.0	-3.8		8.82	-2631.579 04	4.53	
Cbl(III)-H ₂ O, 2	-2631.202 12	322.0	-23.0			-2631.418 97		
Cbl(II)-NO, 3	-2684.644 18	309.2	+66.6	+72.4	6.30	-2684.693 97	4.45	+66.6
Cbl(III)-NO, 4	-2684.411 54	308.6	-1.8	+21.2		-2684.529 58		+71.7
H ₂ O	-76.010 75	14.4				-76.020 88		
NO	-129.247 88	3.2				-129.245 75		
MP2/6-31G(d)/(PW91/6-31G(d)) ^c								
	MP2	E_{BIND}^a (vacuum)	$E_{exchange}^b$ (vacuum)	IP (vacuum)	PMP2	E_{BIND}^a (vacuum)	$E_{exchange}^b$ (vacuum)	IP (vacuum)
Cbl(II)	-2559.261 50 (-2559.501 20) ^d			2.55 (9.07) ^d	-2559.404 84			6.45
Cbl(III)	-2559.169 50							
Cbl(II)-H ₂ O, 1	-2635.696 79 (-2635.712 62) ^d	-148.8 (-3.4) ^d		7.56	-2635.710 29	-66.8		7.93
Cbl(III)-H ₂ O, 2	-2635.423 67	-33.2				-33.2		
Cbl(II)-NO, 3	-2689.061 05 (-3.4) ^d	-153.8 (+4.3) ^d	-5.0	8.95		-58.1	+8.7	8.42
Cbl(III)-NO, 4	-2688.732 52 (-2688.768 04) ^d	-6.1 (+20.3) ^d	+147.7 (+12.9) ^d		-2688.752 11	-12.6	+20.6	
H ₂ O	-76.196 73							
NO	-129.551 41 (-129.566 16) ^d				-129.560 63			

^a The calculated energy for the reaction cobalamin + X → cobalamin-X (X = H₂O or NO), corrected to 0 K using the unscaled zero-point energies.

^b The calculated energy for the reaction cobalamin-H₂O + NO → cobalamin-NO + H₂O, corrected to 0 K using the unscaled zero-point energies. ^c MP2 energies corrected using the unscaled PW91 zero-point energies. ^d ROMP2.

Results and Discussion

Cobalamin has a structure similar to that of porphyrins. However, it lacks one of the bridging methylene groups in the tetrapyrrolic ring, which makes its corrin ring smaller and more flexible because of the partial lack of aromaticity.²⁴

In reduced cobalamin, where Co(II) is 5-coordinate, the ring is tilted upward.

As seen in Figures 1–3, these tilted structures are hardly affected by the binding of NO and H₂O to the Co(II) and Co(III) metal centers. This behavior may indicate that the shape of the cobalamin (or corrin ring) does not play an

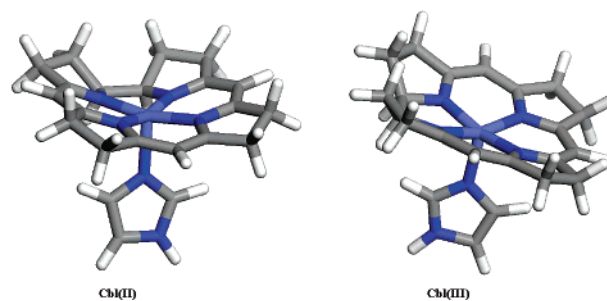


Figure 1. PW91/6-31G(d)-optimized structures of the model compounds Cbl(II) and Cbl(III).

important role in the addition or exchange reactions of NO and H₂O ligands. Table 1 shows the calculated energies.

As seen from Figure 1 and Table 1, there are very significant differences in the calculated bond energies, even between the two DFT methods. As expected, the absolute values of Hartree–Fock and MP2 bond dissociation energies are not reasonable and even the calculated ligand-exchange

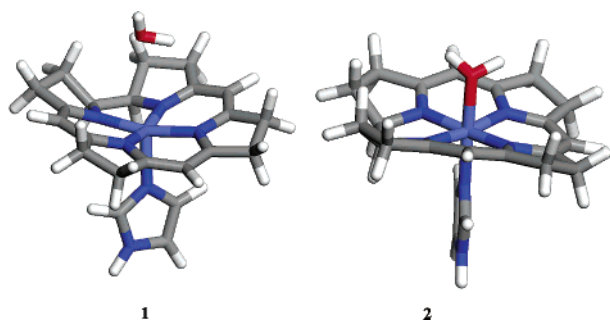
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Table 2. Selected Bond Lengths (Å) and Angles (deg) for Cobalamin Complexes

	B3LYP	PW91	HF	B3LYP	PW91	HF	B3LYP	PW91	HF	B3LYP	PW91	HF
	Co–N(imidazole)			Co–OH ₂								
Cbl(II)–H ₂ O, 1	2.169	2.088	2.302	3.433	3.334	3.468						
Cbl(III)–H ₂ O, 2	1.910	1.882	2.089	2.043	2.042	1.976						
	Co–N(imidazole)			Co–N						N–O	Co–N–O	
Cbl(II)–NO, 3	2.196	2.227	2.103	1.867	1.847	1.916	1.185	1.193	1.184	119.6	120.7	118.0
Cbl(III)–NO, 4	1.927	1.996	1.936	2.081	1.865	3.352	1.149	1.167	1.124	127.4	129.9	117.1

energies (for reactions that are not isodesmic but should nevertheless profit from some error cancellation) deviate very significantly from both DFT methods. The agreement between the B3LYP and PW91 hydration energies in aquacob(II)alamin (−4.9 and −6.9 kcal mol^{−1}, respectively) and aquacob(III)alamin (−19.5 and −23.8 kcal mol^{−1}, respectively) is reasonable. However, the two DFT methods differ very significantly for the NO-binding energies in cob(II)alamin–NO (−3.3 and −30.6 kcal mol^{−1} for B3LYP and PW91, respectively) and for cob(III)alamin–NO (−8.9 and −23.9 kcal mol^{−1}). Although our current results clearly do not allow us to judge the accuracy of any one of the calculational levels, we can extrapolate from the known problems that B3LYP exhibits for the Co–C C bond.^{13,17} We base the remainder of the discussion on the PW91 results as these give higher bond-dissociation energies than B3LYP for all ligands, suggesting that PW91 does not suffer from the known B3LYP weakness in this respect. The general conclusions, however, remain the same whichever functional is used, although the magnitudes of the effects found differ widely.

The interactions between Co(II) and H₂O are weak (**1**; Figure 2), so that replacing the water ligand ought to be

**Figure 2.** PW91/6-31G(d)-optimized structures of the Cbl–H₂O complexes Cbl(II)–H₂O, **1**, and Cbl(III)–H₂O, **2**.

kinetically favorable. The different calculational methods, however, disagree significantly about the nature and strength of the bond between Co and NO in **3** and **4**. The gas-phase binding energy in the latter is calculated to be 5.6 kcal mol^{−1} more negative for **4** than for **3** at B3LYP but 5 kcal mol^{−1} lower at PW91. The binding between cob(II)alamin and NO is treated particularly poorly at the Hartree–Fock level, where the complex **3** is found to be unbound by almost 3 eV despite it having a reasonably short Co–NO distance. This problem is caused by the fact that two open-shell species combine to give a singlet complex, for which Hartree–Fock theory sometimes gives a positive bond energy. In contrast to B3LYP ($\langle S^2 \rangle = 0.76$) and PW91 ($\langle S^2 \rangle = 0.77$), the UHF wave function for cob(II)alamin exhibits strong spin con-

Table 3. NO-Stretching Frequencies of Free NO Species and Cobalamin Complexes Calculated at the B3LYP/6-31G* Level

species	B3LYP		PW91	
	freq (cm ^{−1})	bond length (Å)	freq (cm ^{−1})	bond length (Å)
NO ⁺	2479	1.073	2366	1.084
NO	1991	1.159	1905	1.170
NO [−]	1467	1.276	1375	1.288
Cbl(II)–NO, 3	1733	1.185	1679	1.193
Cbl(III)–NO, 4	1941	1.149	1792	1.167

tamination ($\langle S^2 \rangle = 2.98$) caused by spin states higher than quartet ($\langle S^2 \rangle$ increases to 6.58 on quartet annihilation) but the energetic effect is not large (the restricted open-shell Hartree–Fock energy for cob(II)alamin is only 0.001 hartree less negative than the UHF energy). The consequences of this spin contamination for the UMP2 energies are, however, very significant. The adiabatic UMP2/6-31G(d)/PW91/6-31G(d) ionization potential of cob(II)alamin is only 2.55 eV compared with 9.54 eV at the Hartree–Fock level and 8.87 and 8.60 eV with B3LYP and PW91, respectively. Quartet spin annihilation for the reference function helps somewhat (the PMP2 ionization potential is 6.45 eV) but cannot cure the problem completely because of the contributions from higher spin states than quartets. As found previously by Radom and his group for calculations on organic radicals and their reactions,³⁰ of the Hartree–Fock-based methods, restricted open-shell MP2 (ROMP2) gives by far the most reasonable results for the systems considered here. The ROMP2 results are in general fairly similar to those obtained with B3LYP.

The gas phase DFT-binding energies indicate that displacing water by NO in cob(II)alamin is strongly exothermic (−22.8 kcal mol^{−1}) for the PW91 functional but slightly endothermic (+1.6 kcal mol^{−1}) for B3LYP. The SCRf calculations within a simulated water environment show a significant solvent effect. The binding situation is reversed for B3LYP: replacing water by NO is now favored by 1.5 kcal mol^{−1}. The effect of solvation on the exchange energy using PW91 is the reverse: replacing water by NO is roughly 9 kcal mol^{−1} less endothermic in water than in the gas phase. In this case, however, both calculations indicate that NO can replace water. The exchange reaction is less sensitive to solvation for cob(III)alamin. Water solvation disfavors NO binding by 1–4 kcal mol^{−1}, and the two functionals agree that cob(III)alamin cannot bind NO in an aqueous environment. Thus, the calculations indicate clearly that cob(III)alamin cannot bind NO in aqueous solution but that reduced cob(II)alamin can. However, the reasons are different

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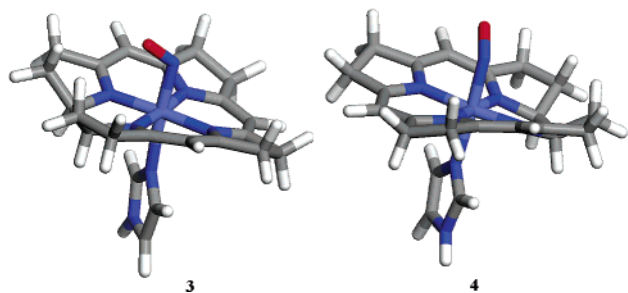
Table 4. Calculated Charges on Selected Atoms of Cobalamin–NO Complexes Calculated at B3LYP/6-31G*, HF/6-31G*, and HF/6-31G*/B3LYP/6-31G*

	atom	B3LYP		PW91		HF/HF		HF//B3LYP	
		Mulliken	NPA	Mulliken	NPA	Mulliken	NPA	Mulliken	NPA
Cbl(II)–NO	Co	0.990	1.341	0.919	1.306	1.550	1.841	1.537	1.814
	N	0.012	0.017	0.003	0.002	–0.090	–0.163	–0.072	–0.145
	O	–0.172	–0.247	–0.139	–0.211	–0.329	–0.405	–0.323	–0.395
	NO	–0.160	–0.230	–0.136	–0.209	–0.419	–0.568	–0.395	–0.540
	N _{im}	–0.460	–0.584	–0.426	–0.536	–0.672	–0.685	–0.658	–0.681
Cbl(III)–NO	Co	1.072	1.447	0.964	1.350	1.737	2.025	1.473	1.493
	N	0.138	0.159	0.107	0.124	0.171	0.249	0.172	0.248
	O	–0.016	–0.066	–0.038	–0.070	–0.144	–0.241	–0.151	–0.222
	NO	0.122	0.093	0.079	0.054	0.027	0.008	0.021	0.026
	N _{im}	–0.485	–0.549	–0.454	–0.538	–0.727	–0.709	–0.635	–0.623

for the two functionals. PW91 suggests that NO can replace water at a cob(II)alamin center both in the gas phase and in water, whereas B3LYP indicates that this preferential binding of NO over water is a solvent effect.

Some selected geometrical parameters are given in Table 2. In aquacob(II)alamin, **1**, H₂O is loosely bound to the metal and the Co–O distance is between 3.334 (PW91) and 3.468 Å (HF). Surprisingly, the other axial ligand imidazole is also bound relatively loosely with Co–N bond lengths between 2.088 (PW91) and 2.302 Å (HF). The situation is reversed in aquacob(III)alamin, where both axial ligands are tightly bound, resulting in shorter and stronger bonds (Table 2). The bond-length variations cannot be rationalized as the result of competition between the two axial ligands in **1** but are rather consequences of the methods used. Both axial bond lengths are shortest with PW91 and longest with HF, the B3LYP values being intermediate.

The Co–NO fragment has a bent structure in both Cob(II)alamin–NO, **3**, and Cob(III)alamin–NO, **4** (Figure 3),

**Figure 3.** PW91/6-31G(d)-optimized structures of the Cbl:NO complexes Cbl(II)–NO, **3**, and Cbl(III)–NO, **4**.

although there are once again significant differences between the different methods for **4**. There is, however, relatively good agreement on the geometry of the cob(II)alamin complex **3**, for which the Co–NO bond is calculated to be 1.82–1.92 Å long and the Co–N–O angle between 118 and 121°. The N–O bond length is increased to 1.18–1.19 Å (from 1.15 Å in **4**), whereas the distance between Co and the bound imidazole nitrogen is 2.1–2.2 Å. This trend is reversed in nitrosocob(III)alamin. The Co–NO distance increases and Co–N(imidazole) decreases significantly at B3LYP and slightly at PW91. These results indicate weaker binding of NO to the Co(III) center. It is worth noting that HF/6-31G* shows a very weak interaction (Co–NO = 3.352 Å) between NO and the Co(III) center. In fact, there is no

evidence in the literature that the Co(III)–NO complex exists, in accord with our result.^{1b} In contrast, binding of NO to the five-coordinate Cob(II)alamin results in the formation of a six-coordinate nitrosocob(II)alamin complex in which the bond trans to NO is weakened as shown by the increase in pK_a value of the dimethylbenzimidazole ligand.⁴ These results are consistent with the calculated geometries despite the large differences between the three different calculational methods.

A comparison of NO bond lengths and NO stretching frequencies (shown in Table 3) in NO complexes of cobalamin, the free NO radical, cation, and anion, suggests that NO has radical character in the Cob(III)alamin–NO complex, **4**, whereas it is partially anionic in the cob(II)alamin–NO complex, **3**. This provides evidence for an electron transfer from the Co(II) center to the NO to give Co(III)–NO[–], as has been suggested by experimental results on the basis of resonance Raman and ¹⁵N NMR spectroscopy.^{3,4} These results are essentially independent of the functional used. However, the formal (natural population analysis, NPA³¹) charge on NO in **3** is only –0.23 or –0.21 e[–] (B3LYP and PW91, respectively), far from indicating that the NO is bound as an anion (Table 4). Density functional theory tends to underestimate charge transfer and polarity^{32,33} so that an electron transfer would not necessarily be evident in the DFT results. We have therefore used the HF/6-31G* results to be able to characterize the nature of the NO complexes because DFT is known to underestimate charge separation in comparison to Hartree–Fock.³⁴

Table 4 shows the NPA and Mulliken charges calculated at both levels. The HF calculations give a charge of –0.57 on the NO group in the Cbl(II)–NO complex, compared with essentially zero for the corresponding Co(III) complex. These results suggest that the Co(III)–NO[–] bonding model is

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appropriate, especially as the HF charges on NO in the Cbl(III)–NO complex are very close to zero. However, the NO is dissociated from the Co center in the HF geometry of this complex, so that the charge separation may be artificially small. We therefore performed single point HF calculations on the B3LYP/6-31G* geometries to rule out a purely geometrical effect. The results show essentially the same charge distributions as found for the HF-optimized geometries (see Table 4).

Conclusions

Our calculations on model systems support the proposal that cob(II)alamin can bind NO in aqueous solution but that cob(III)alamin cannot. The reason for this behavior varies, however, with the DFT method used.

B3LYP suggests that the reason for the experimental observations lies less in the abilities of the two oxidation states to bind NO than in their complexation energies to water as a sixth ligand. Cbl(II) (complexation energy -3.3 kcal mol $^{-1}$) is calculated to bind even less strongly to NO in the gas phase than Cbl(III) (complexation energy -8.9 kcal mol $^{-1}$). However, in competition with water, which binds strongly to Cbl(III) (complexation energy -19.5 kcal mol $^{-1}$) but weakly to Cbl(II) (complexation energy -4.9 kcal mol $^{-1}$) in the gas phase, NO complexation is more favorable for Cbl(II). The calculated solvent effects in simulated water solution tip the bonding equilibrium in favor of NO binding for Cbl(II) but not for Cbl(III).

The PW91 functionals give a simpler and probably more reliable picture. In this case, the inherent ability of cob(II)alamin to bind NO is higher than that of cob(III)alamin by about 5 kcal mol $^{-1}$ in the gas phase. This, combined with the fact that cob(II)alamin coordinates about 17 kcal mol $^{-1}$ more strongly to water than cob(III)alamin, results in a 22 kcal mol $^{-1}$ preference for NO binding over hydration for cob(II)alamin in the gas phase. The gas-phase

exchange reaction is thermoneutral for cob(III)alamin. In this case, simulation of the bulk solvent has the effect opposite of that found with B3LYP in that it disfavors NO binding to cob(II)alamin by about 10 kcal mol $^{-1}$.

The calculated vibrational frequencies support the idea that the Cbl(II)–NO complex is best described as Cbl(III)–NO $^{-}$, although the charge transfer from metal to NO is hardly evident in the DFT population analyses. This preference for covalent bonding in B3LYP calculations has been noted previously³² but seems to be particularly evident in this example. Perdew and Ernzerhof³³ have pointed out that this weakness is a direct consequence of the self-interaction error. Hartree–Fock calculations support the charge-transfer interpretation of NO binding to Cbl(II) but are completely unreliable for calculating energies.

The extreme dependence of the results on the density functionals found in this work has been inferred in previous studies on the Co–C bond strength in cobalamin model compounds^{13,17} by comparison of the experimental and theoretical results but has not been investigated systematically. Our results for the Co–NO bond strength suggest that the PW91 functional performs better than B3LYP, but this conclusion must remain tentative until comparison studies with nonhybrid functionals have been performed for the Co–C bond problem.

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