Theoretical Investigation of Steric and Electronic Effects in Coenzyme B_{12} Models

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In this study, the first density functional theory calculations on B_{12} models that contain the entire corrin ring are presented to evaluate earlier findings by other groups. Eight octahedral corrin systems with various axial ligands have been subject to a full density functional theory (DFT) lacvp** geometry optimization in vacuo. The largest of these calculations included around 1000 basis functions. For energies of optimized structures, we increased the basis set to a triple-ζ valence type. The effects of the different axial substituents on the crucial Co−C bond length and the corrin folding have been evaluated. We find a systematic cis-steric effect and a less systematic trans induction. The corrin framework is fairly inert toward the size of the axial R-ligands, which argues against a mechanochemical trigger mechanism. The HOMO−LUMO gap increases through the steric series via a lowering of the HOMO energy, making the corrins less susceptible to homolytic cleavage. Rather different equilibrium structures of adenosylcobalamin and methylcobalamin models are anticipated from this study, which also indicates that the former has ca. 5 kcal/mol higher HOMO and LUMO energy. This is a theoretical explanation why adenosylcobalamin is more easily homolyzed, whereas methylcobalamin is heterolyzed.

Introduction

Some of the most extraordinary organometallic biomolecules and probably the ones attracting the most attention from researchers everywhere are the cobalamins or B_{12} coenzymes, which have fascinated chemists for decades. The cobalt-containing B_{12}-coenzymes are the active forms of vitamin B_{12}, which is also known as cyanocobalamin (see Figure 1). The cobalamin contains a planar framework, called the *corrin ring* (from *core*),[1] which contributes the four equatorial nitrogen ligands to the cobalt ion in the center. The corrin system is somewhat similar to the porphyrin system except that (i) one of the bridging methine groups is missing and (ii) the corrin ring is partially saturated, containing pyrrolines instead of pyrroles,[1] thus reducing the planarity. Seven amide chains are placed on the corrin ring. The *nucleotide loop* connects one of these chains with the α-axial ligand, which is 5,6-dimethylbenzimidazole (DMB). Here, the α-ligand is denoted L, while the trans axial group (the *β*-ligand) is denoted R.

The two biologically active forms of cobalamins are adenosylcobalamin ($AdoB_{12}$) with an R = 5′-deoxyadenosyl group instead of the cyano group and methylcobalamin (MeB_{12}) with R = methyl. Methylcobalamin is the cofactor of methylating enzymes such as homocysteine methyl transferase (methionine synthase), which is essential for DNA and methionine synthesis. Adeno-

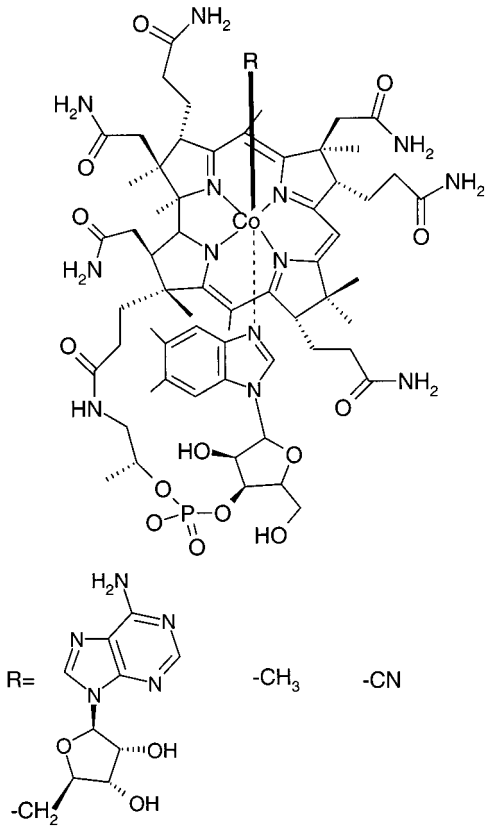

Figure 1. Cobalamin system.

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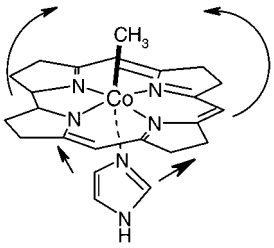

Figure 2. Mechanochemical trigger mechanism.

sylcobalamin is coenzyme for isomerases, for example, methylmalonyl-CoA mutase, which catalyzes some alkyl migration reactions.[1,2] Vitamin B_{12}-deficiency results in megaloblastic anemia (because of MeB_{12}-deficiency) and neurological degeneration (because of $AdoB_{12}$-deficiency).

It is now believed that $AdoB_{12}$ acts as a radical generator through homolytic cleavage of the cobalt−carbon (Co−C) bond. Under the influence of the enzyme and substrate, the coenzyme separates into two radicals, followed by the radicalization and methyl migration of the nearby substrate. The isomerized product is obtained by hydrogen addition to the substrate.[1,3,4] MeB_{12}, on the other hand, is probably facilitated to cleave the Co−C bond heterolytically.[4] It is now obvious that both coenzymes exhibit major conformational changes during binding to their enzymes, changes that probably are important means of weakening the Co−C bond. It has recently been suggested that MeB_{12} is active in a base-off form, in which the nucleotide loop is turned away toward a hydrophobic domain in the apoenzyme. In crystal structures of MES,[5] MCAM,[6] and glutamate mutase,[7,8] a histidine residue of the enzymes is ligating cobalt. Sequence similarities in all cobalamin-dependent enzymes, the conserved sequence Asp-X-His-X-X-Gly, have made Ludwig et al. suggest that such base-off mechanisms are common.[3,5] The discovery of an active base-off form of MeB_{12} with a histidine residue coordinating cobalt has provoked further work on model systems with imidazole as the α-axial ligand to investigate the binding properties of the histidine.[9−11]

The so-called *mechanochemical* trigger mechanism (Figure 2) has been invoked to explain how homolysis might be achieved in $AdoB_{12}$.[12−14] It involves compression of the Co−N_{ax} bond, resulting in steric interactions of DMB with the corrin ring and the nucleotide loop. This is an α-side trans-steric effect. Such compression would increase the electron density on cobalt and thus weaken the β-axial Co−C bond because of less σ-donation by R into the bonding orbitals. This is a trans induction. Hence, the mechanochemical trigger mechanism involves a combination of two effects. Recent Raman studies by Marzilli's group show corrin distortion in enzyme-bound $AdoB_{12}$,[15] so the corrin folding (butterfly bending) seems to be an important step toward Co−C bond cleavage. Such a mechanism is more likely to be a cis steric effect, a Co−C bond lengthening as a result of steric interactions between corrin ring + amide chains and the β-axial ligand.

Kräutler et al. have compared the crystal structure of $AdoB_{12}$ with the structure of cob(II)alamin, which is the intermediate that arises from homolytic cleavage of the β-axial bond.[16,17] They found that the two structures are very similar, indicating that it is not just a distortion of the corrin moiety that leads to the homolysis products but rather strong stabilization of the radicals by the environment (apoenzyme + substrate). The similarity in structure also suggests that the activation barrier of the homolysis reaction is low.

Theoretical investigations could be an important part of coenzyme B_{12} research. A force field has been developed and applied by Brown, Marques, and co-workers,[18,19] which is specifically designed to do molecular mechanical calculations on cobalamins and which has recently been extended to cobaloxime B_{12} models as well.[20,21] However, these calculations, although important for conformational searching studies, will probably not elucidate the mechanisms of coenzyme B_{12}-supported reactions since electronic effects cannot be accounted for in most force fields. Theoretical investigations on relative bond-breaking propensities require quantum mechanics. Until now, only a very few quantum mechanical calculations have been carried out on the cobalamin model systems because of their somewhat large size. The early work by Christianson and Lipscomb could be considered the pioneer computational study in this field,[22] but the triaminomethylcobalt(III)-amide system is of course only of historical relevance in modern B_{12} chemistry. Semiempirical calculations have been done on cobaloxime models as well as the much more realistic corrin models[23−25] first introduced by Zhu and Khostic[26] (see Figure 2). These studies have been important in the discussion of the balance between

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steric and electronic effects during reaction. However, semiempirical methods are inherently not very good for achieving equilibrium structures. PM3(tm) was shown to have an average error of around 0.05 Å for Co−C bond lengths and above 0.1 Å for Co−N(axial) bond lengths,[25] whereas PRDDO was around 0.05 Å for all bond lengths.[23,24] DFT calculations on such model systems is expected to provide higher accuracy. Very recently, Morokuma and co-workers used DFT to calculate nuclear quadropole couplings of simple coenzyme B_{12} models without a planar framework.[27] The study aimed at describing the axial nitrogen base, which was also modeled in solution. Experiments from the same group proved these DFT calculations to be fairly accurate.[28] In the current study, the B3LYP method is used to evaluate the equilibrium structures of corrin models with different R-groups in the same spirit as earlier model studies. The main problem of the hitherto used force field approach is that the parameters either are based on relatively few data points[18,19] or are parametrized for cobaloximes,[20] which are now known to have a much too flexible equatorial framework to mimic the corrin folding.[24] The latter mode is an essential part of the reaction mechanism, and hence, we argue that the corrin ring is essential in model studies. Since both the level of theory and the models used in this study are of quite good quality, it is the highest level of theoretical guess on cobalamin equilibrium structures and energetics so far presented in the literature.

Experimental Section

General Methods. The DFT calculations were done with the JAGUAR program.[29] We used the Becke-3 exact exchange parameter functional.[30−32] This hybrid functional has been found to be one of the most efficient DFT functionals for obtaining good equilibrium structures as well as properties such as harmonic vibration frequencies.[33] The DFT methods also have the best cost−benefit ratio of all methods since they scale approximately as the analogous but cruder Hartree−Fock equations.[34] This is also true for inorganic compounds, in which correlation may be dealt with in a cheap way by using DFT.[35] The Lee−Yang−Parr (LYP) local + nonlocal correlation functional was used together with the Vosko−Wilk−Nusair (VWN) functional (B3LYP).[36]

For geometry optimization, we used the lacvp** basis set,[37−39] which includes effective core potentials for cobalt alone. lacvp is a valence + outermost core-basis, so cobalt(III) is treated with an effective neon core and includes the m-shell (with six d-electrons and the 3s,3p electrons) in the total basis. The basis set used for all other atoms is the Pople style 6-31G basis (double-ζ valence), so except for cobalt, the calculation is basically as with a 6-31G** basis. The version of JAGUAR used here is only suitable for closed-shell systems,[29] and the octahedral cobal(III)amins as well as the +1-charged corrin models are closed-shell d^6 configurations. Optimization was done without constraints (full optimization). The single-point energies of the optimized structures were calculated at the triple-ζ valence B3LYP/lacv3p** level (corresponding to a 6-311G** basis set for everything except cobalt).

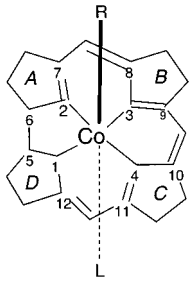

Figure 3. Structure and nomenclature of the corrin model system used in this study.

Optimized Model Systems. The nomenclature used in the corrin systems is shown in Figure 3. Eight corrin model systems have been fully geometry optimized: aminomethyl-corrin^{+1} (**1**), imidazolmethyl-corrin^{+1} (**2**), methyl-5,6-dimethylbenzimidazolcorrin^{+1} (**3**), ethyl-5,6-dimethylbenzimidazolcorrin^{+1} (**4**), isopropyl-5,6-dimethylbenzimidazolcorrin^{+1} (**5**), *tert*-butyl-5,6-dimethylbenzimidazolcorrin^{+1} (**6**), cyano-5,6-dimethylbenzimidazolcorrin^{+1} (**7**), and 5′-deoxyadenosyl-5,6-dimethylbenzimidazol-corrin^{+1} (**8**) (see Figure 4). All model systems are cationic; the phosphate moiety which renders the full B_{12} neutral was not incorporated in our models. Our intention was that the varying size of R would probe the steric control of the carbon−cobalt bond strength, whereas calculations employing the cyano group should probe for electronic effects. The equilibrium structures obtained should give an indication of what steric and electronic effects might be active in a vacuum. Solvation calculations are of course the next step, which will be approached in future work. Here, we only consider relative energies and equilibrium structures, while transition state considerations will be postponed to a later time.

The number of basis functions used for geometry optimization were rather large: 507 basis functions for **1**, 568 basis functions for **2**, 682 for **3**, 706 for **4**, 730 for **5**, 754 for **6**, 681 for **7**, and 965 basis functions for **8**. The cpu-hours/iteration step were between 2.5 and 6.1 h (on R10K SGI workstations), increasing with the size of the molecule. These are impressively low figures, considering the number of basis functions and the full optimization task. For the lacv3p** basis sets, the number of basis functions was approximately 25% larger, the largest (**8**) having 1225 basis functions.

Results and Discussion

The DFT HOMO and LUMO energies are presented in Table 1. We compare these energies in the frozen-orbital approximation (assuming that the derivatives of the overlaps are zero in the Fukui equations). The optimized geometries are presented in Table 2 (bond lengths) and Table 3 (torsion angles). The measured bond lengths are the Co−C bond, the Co−N_{ax} bond, and each of the four Co−N(corrin) bonds. The torsion angles measured are the eight possible cis-equatorial N−Co−N−C torsion angles. They have a significant importance, since they measure the degree of distortion of the corrin moiety as a function of their respective axial R-ligands. They are defined in the following way: $\omega_a = \omega$(N3−Co−N2−C7), $\omega_b = \omega$(N3−Co−N4−C10), $\omega_c = \omega$(N4−Co−

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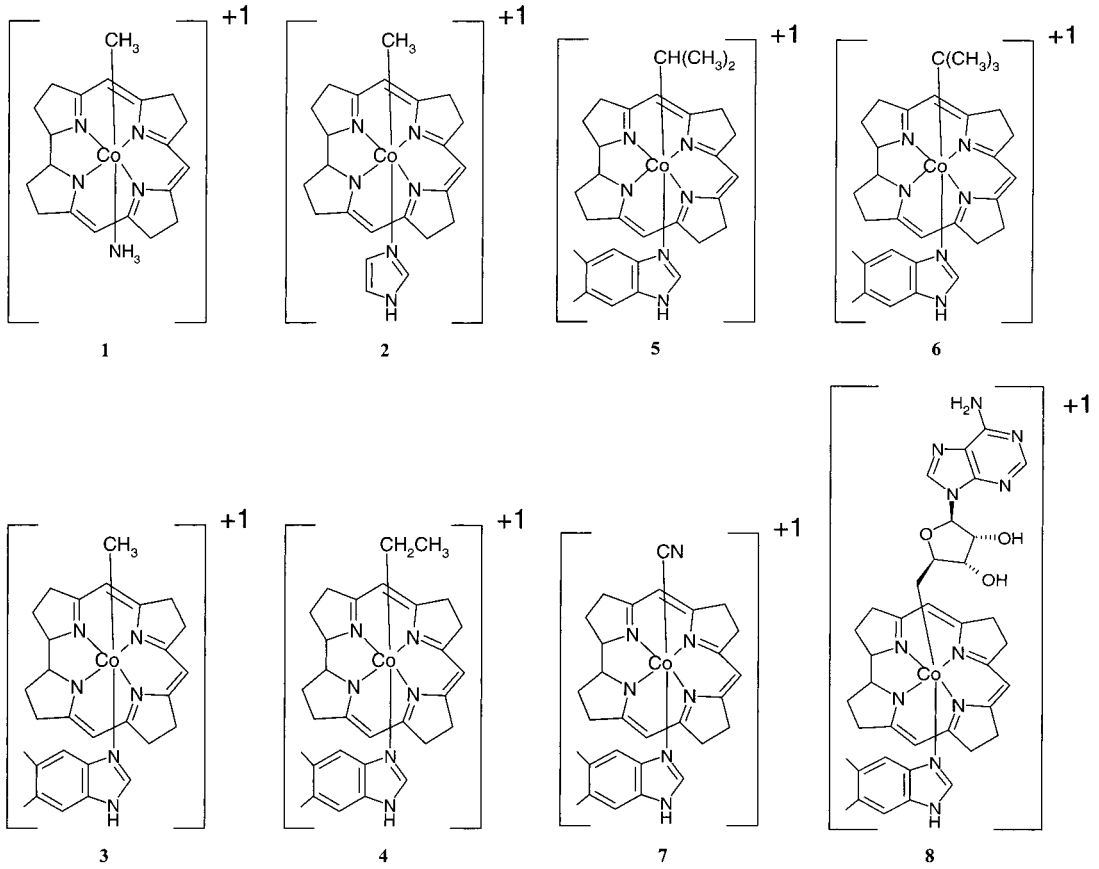

Figure 4. Different coenzyme B_{12} models optimized in this study.

Table 1. B3LYP/lacv3p Energies Obtained for Model Systems in This Study**

energy	1	2	3	4
E_{HOMO} (au)	−0.3109	−0.3035	−0.3037	−0.3026
E_{LUMO} (au)	−0.1891	−0.1817	−0.1813	−0.1809
$E_{HOMO-LUMO}$ (a.u)	−0.1218	−0.1218	−0.1224	−0.1216
$E_{HOMO-LUMO}$ (kcal/mol)	76.44	76.46	76.81	76.32
energy	5	6	7	8
E_{HOMO} (au)	−0.3078	−0.3079	−0.3158	−0.2951
E_{LUMO} (au)	−0.1816	−0.1815	−0.1914	−0.1736
$E_{HOMO-LUMO}$ (au)	−0.1262	−0.1264	−0.1244	−0.1215
$E_{HOMO-LUMO}$ (kcal/mol)	79.18	79.31	78.03	76.23

Table 2. B3LYP/lacvp Equilibrium Bond Lengths (Å) Obtained in This Study**

bond length	1	2	3	4	5	6	7	8
r(Co−C)	1.966	1.962	1.967	2.001	2.051	2.110	1.862	1.994
r(Co−N_{ax})	2.269	2.283	2.332	2.345	2.569	2.773	2.190	2.335
r(Co−N1)	1.846	1.900	1.902	1.897	1.896	1.899	1.902	1.896
r(Co−N2)	1.903	1.894	1.896	1.901	1.890	1.897	1.893	1.893
r(Co−N3)	1.946	1.949	1.946	1.945	1.946	1.944	1.952	1.938
r(Co−N4)	1.954	1.946	1.941	1.940	1.947	1.945	1.949	1.947

N3−C9), $\omega_d = \omega$(N4−Co−N1−C12), $\omega_e = \omega$(N1−Co−N4−C11), $\omega_f = \omega$(N1−Co−N2−C6), $\omega_g = \omega$(N2−Co−N1−C5), and $\omega_h = \omega$(N2−Co−N3−C8). The numbering system used for the corrins is shown in Figure 3. The average of the unsigned torsion angles is given as a measure of overall out-of plane corrin folding in each species.

The final equilibrium structure of **3** should resemble the structure of MeB_{12}, whereas **8** is the obvious model of $AdoB_{12}$. All investigated cobalamins have equatorial Co−N distances around 1.90 ± 0.04 Å, independent of the axial ligands.[1] The crystal structure of MeB_{12} has

Table 3. B3LYP/lacvp Equilibrium Torsion Angles (deg) Obtained in This Study**

torsion angle	1	2	3	4	5	6	7	8
ω_a	−11.5	−13.0	−11.8	−11.9	−9.6	−11.2	−13.5	−12.5
ω_b	1.6	−0.6	−2.4	−1.8	0.1	−2.5	−4.8	−4.1
ω_c	−2.6	−1.4	0.7	0.0	−1.9	0.0	2.7	2.9
ω_d	−6.0	−6.4	−0.5	−3.1	−4.3	−2.8	1.7	1.5
ω_e	5.2	4.1	−0.6	0.6	1.0	−2.6	−2.2	−4.5
ω_f	−10.0	−11.1	−13.2	−14.2	−12.7	−13.5	−12.3	−13.9
ω_g	−14.8	−13.8	−12.5	−10.7	−11.8	−11.6	−13.7	−10.9
ω_h	10.3	10.5	12.3	13.0	10.3	14.4	13.5	13.5
$\omega_{average}$	7.8	7.6	6.8	6.9	6.5	7.3	8.1	8.0

r(Co−C) = 1.99 Å and r(Co−N_{ax}) = 2.19 Å.[40] The Co−C bond length difference between crystal structure and DFT is insignificant, about 0.02 Å. The difference of 0.14 Å between r(Co−N_{ax}) in **3** (see Table 3) and MeB_{12} may be due to either (i) the insufficient model system, especially the effect of the nucleotide loop, (ii) shortcomings of the theory and level of calculation, or (iii) the fact that the models are optimized in vacuo. Of these three effects, the first could be evaluated if analogous DFT calculations could be done on MeB_{12}. This is currently beyond our computational resources because of the size of the coenzyme. Hence, the importance of the nucleotide loop is only possible to evaluate by force field, semiempirical, or QM-MM methods. In a recent PM3(tm) study, the introduction of the loop and amide chains was found to have minor influence on axial bond lengths.[25] We are currently trying out the two other approaches as well. However, high-level QM/MM is

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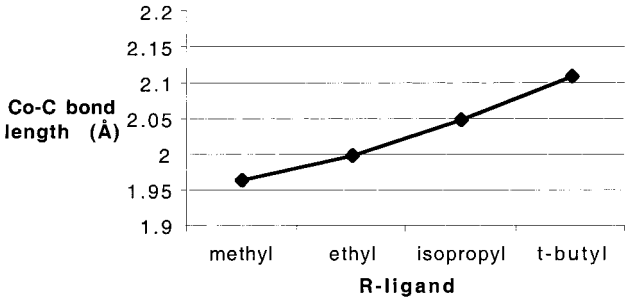

Figure 5. Cis-steric effects in corrins: The Co−C bond distance as a function of axial ligand size.

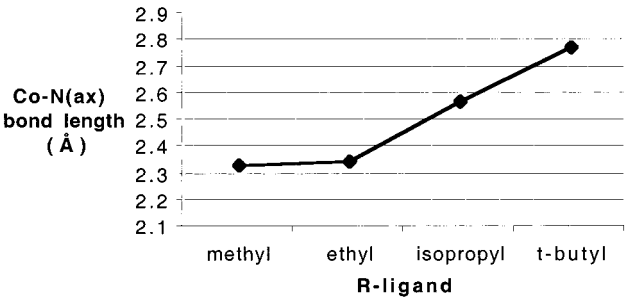

Figure 6. Trans-steric effects in corrins: The Co−N_{ax} bond distance as a function of trans-axial ligand size.

exceedingly difficult to use on these four-ring systems if link regions are to be introduced.

The accuracy level of the current B3LYP/lacv3p**: B3LYP/lacvp** method is quite high (better than large basis Hartree−Fock) and includes both polarization and correlation effects. However, B3LYP is known to underestimate the strength (overestimate the length) of dative bonds to metals.[41] Shortcomings due to solvation effects or crystal packing forces may change the absolute numbers to some extent, and especially folding of the corrin ring may be different in aqueous solution and enzyme environments. Nevertheless, one could argue that the inherent energetics of conformational changes in the coenzyme *should* be modeled in a vacuum since the relative importance of enzyme-bound, solvated, and crystal conformations cannot be separated. True equilibrium structures and conformations for use in reaction considerations should ideally be modeled within an enzyme-like environment. The isolated (i.e., in vacuo) steric and electronic effects are however our sole aim in this study.

One of the immediate conclusions to be drawn from the trends in Table 2 is that the equatorial Co−N distances are fairly constant in all model systems. This indicates that cobalt is immobile regardless of the changes in axial ligands; that is, the internal structure of the corrin−cobalt part is preserved during all the changes induced here. On the other hand, it is seen that the Co−C bond lengths of the molecules with R = methyl (**1**, **2**, **3**), ethyl (**4**), isopropyl (**5**), and *tert*-butyl (**6**) directly reflect the steric strain of the corrin ring with the axial R-ligand. For the DMB ligand, the Co−C bond lengths increase from 1.967 Å with R = methyl (**3**), to over 2.001 Å with R = ethyl (**4**), to 2.051 Å with R = isopropyl (**5**), which is an elongation of 0.08 Å. The introduction of yet another methyl group in the β-ligand again increases the Co−C bond length by approximately 0.06 Å in **6**. This trend is a much more convincing indication of a cis-steric effect than obtained earlier by PRDDO methods.[19] The cis-steric effect in corrins is illustrated in Figure 5.

The torsion angles of the series **3**−**6** show that these molecules have rather similar corrin folding, although one or two dihedrals vary around 4° as a function of these β-ligands. That is, the observed differences in Co−C bond length is not accompanied by a large distortion of the corrin ring. However, the length of the trans Co−N_{ax} bond to the DMB ligand increases drastically as a function of the β-ligand size. The Co−N_{ax} bond length grows by 0.24 Å by adding two methyl groups and by another 0.20 Å by adding one more methyl group. This is a theoretical support of the idea that changes on the β-side of the corrins induce elongation of the trans Co−N bond.[42] The tendency shows that in some limit, beyond for example 3 Å, one would not consider DMB to be coordinating cobalt; that is, the corrin would be in the DMB-off form. This tendency toward a DMB-off form is induced primarily by these changes on the β-side of the corrin. Some of this tendency is probably mediated by the modest changes in the corrin framework (*trans steric influence*), but in contrast to PM3(tm) results, it suggests that some kind of trans induction might be important here together with changes mediated purely by "butterfly bending", because corrin folding is rather modest. The effect is presented here as the combined trans-steric and induction effects, see Figure 6.In the steric series **3**−**6**, the primary effect is an elongation of the Co−C bond, not corrine distortion. In the same series, the ability to form a stable carbocation is also increasing. The series clearly model the inital path of a heterolytic cleavage, with the electrons retained by Co. It could be expected that the increase of electron density on Co would result in raising the HOMO energy. However, for the complexes with the longest Co−C bonds (**5** and **6**), Co is clearly getting closer to the d^8 square-planar state of the heterolysis product, with simultaneous elongation of the Co−N_{ax} bond. The loss of the dative Co−N bond gives the net effect that the HOMO energy actually decreases by 2.5−3 kcal/mol in the series. We also note that the HOMO is *not* a localized $\sigma(sp^3\text{-}d_{z^2})$ type MO (as the LUMO may be),[43,44] but is delocalized over both axial bonds.

In **8**, which is the model of $AdoB_{12}$, the HOMO is destabilized by 5.4 kcal/mol relative to **3**, which mimics MeB_{12}. A higher HOMO energy should correlate with a higher preference for homolytic cleavage, as an electron is more easily lost by Co. In addition, the LUMO is also higher in **8**, making it less susceptible to nucleophilic attack and resulting heterolytic cleavage of the Co−C bond. The corrin rings have rather similar conformations, and the largest deviation is observed in torsion angle ω_e (4°), which describes the folding of rings D and C relative to each other. The different folding in this area is basically a result of electrostatic interaction between the adenosyl and corrin moieties. This may be

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a theoretical explanation of why $AdoB_{12}$ prefers homolysis and a Co(II) intermediate,[45] while MeB_{12} keeps both its electrons during methyl transfer. During heterolytic cleavage of MeB_{12}, both electrons stay on cobalt to produce a square-planar cobalt(I) intermediate.[46,47] It should be noted though that the arguments here are based on ground state effects. For a deeper understanding of the actual reaction in vivo, it may also be necessary to consider true transition state effects, but this is beyond the goals of the current investigation.

The next consideration is if there is an influence from the electronic nature of the $Co-N_{ax}$ bond on the trans Co−C bond, i.e., what we call a α-side trans-induction effect. The $Co-N_{ax}$ bond is 0.05 Å shorter in **2**, where DMB has been replaced by imidazole, than in **3**, indicating an α-side cis-steric *or* electronic effect. However, the difference in the trans-Co−C bond lengths between these two model systems is only 0.005 Å, and except for ω_d and ω_e, where the benzene part of the DMB interacts with the corrin ring, the torsion angles of the corrin moiety are not affected by the different α-ligands. The HOMO and LUMO energies of the two species are quite similar. A histidine group ligates in, for example, $AdoB_{12}$-MCAM holoenzyme with a $Co-N_{ax}$(his) distance of approximately 2.5 Å.[6] B3LYP indicates that there is no particular histidine-on effect.

As kinetic experiments by Finke and co-workers show, one-electron reduction can strongly enhance homolysis of cobalamins because of the very localized Co−C $\sigma(sp^3-d_{z^2})$ type LUMO.[44] Relative changes in the energies of the frontier orbitals should therefore be considered an important contribution toward achieving Co−C bond fission in vivo. B3LYP theory suggests that the effects concentrate on the respective sides of the corrin and do not mix very much, but a trans induction is observed.

The theoretical indication of a weak trans electronic effect is further supported by comparison of **1** to **3** and **7** to **3**, respectively. In **1**, the ammonia ligand allows for more electron donation into empty d-orbitals on cobalt, thereby stabilizing both HOMO and LUMO. The gap is however similar to the imidazole and DMB analogues, **2** and **3**, and considering the axial bond lengths of **1**, **2**, and **3**, one finds no obvious differences in these. Our conclusion is that the trans steric and electronic effects are not separable in this study, but together they are quite important and provide a useful picture of the heterolytic reaction coordinate. We illustrate the combined trans effect in Figure 6 and point out that it is probably mainly a steric effect, although induction properties of the alkyl groups interfere somewhat with this trend.

Compound **7** has a cyano group as β-ligand and is the vitamin B_{12} model. The cyano ligand is a poor σ-donor but a strong π-acceptor. Its electronic properties are thus very different from the methyl ligand. The altered properties of the Co−C bond are reflected in the equilibrium bond length (1.862 Å), which is 0.1 Å shorter than in the methyl analogue **3** due to back-donation to carbon, which gives the bond a higher bond order. This shortening has a profound effect on the trans-axial Co−N bond length, which is reduced to 2.190 Å, about the same percentile decrease in bond length as for the Co−C bond. The corrin folding is much the same as in **3**; the torsional angles differ only by a couple of degrees. Again, this shows a very strong ability of the corrin system to polarize charge in the axial directions. Equatorial distances as well as corrin torsion angles are rather unaffected by these electronic differences, but the two trans effects, the electronic induction and the steric, are inherently connected.

The obvious coupling between axial bond lengths suggests that these trans electronic/steric effects are the means of destabilizing either of the two bonds along the reaction coordinate. Hence, according to these calculations, elongation of the $Co-N_{ax}$ bond should inherently lead to a destabilized Co−C bond. MeB_{12} seems to have a somewhat different reaction coordinate than $AdoB_{12}$, and the latter may in some enzyme environments keep its nucleotide loop as DMB-on. Heterolysis seems to evolve along a coordinate in which both axial bond lengths are increased simultaneously. These destabilizations are afforded by steric strain on both sides or by electronic changes (protonation) of DMB on the α-side.[3,42,48] Steric strain on the β-side has been found in this study to promote the concerted reaction coordinate in a quite general way, since both axial bond lengths are proportional in length as a function of larger R-ligand. This reaction coordinate leads to a square-planar Co(I) intermediate, the one achieved by heterolysis. The group of Banerjee has recently suggested a reaction mechanism of MCAM in which the nucleotide loop is important for facilitating the slow reorganization of the holoenzyme.[49−52] After protonation of the DMB ligand follows a fast coordination of the histidine residue of the active site to the base-off form of the coenzyme.[50] Homolysis would hence, on the other hand, follow several different reaction coordinates, in which the corrin moiety is distorted toward the square-pyramidal structure; that is, upward corrin folding (butterfly bending) should be observed. The Raman experiments by Banerjee et al. suggest that homolysis happens in a nonconcerted way, in which the nucleotide loop is preserved by docking in the apoenzyme. The B3LYP optimizations and energy calculations done in this work support different minimum energy forms of $AdoB_{12}$ and MeB_{12}, since the two equilbrium structures have some differences in corrin conformations and, more important, the electronic nature of the Co−C bonds differ in the two coenzyme models. Special circumstances, such as hydrogen abstraction on the β-side and effects from a catalytic triade on the α-side, must be investigated specifically.

Recent kinetic studies by Finke and co-workers[53] and several other groups[15,49] indicate that a trans electronic effect is absent during homolysis. DFT suggests a strong combined trans steric and induction effect. Hence, we

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want to suggest the above-mentioned two different mechanisms for MeB_{12} and $AdoB_{12}$, which support Marzilli's idea of DMB as a heterolysis-inhibiting ligand and that homolysis of $AdoB_{12}$ is proceeding in a base-on manner.[15] One mechanism is promoted mainly by trans induction, the simultaneous stretching of axial bonds leading to heterolysis of MeB_{12}, and the other is promoted by docking of the nucleotide loop, upward butterfly bending toward a flattened corrin conformation of $AdoB_{12}$, leading to β-side steric repulsion (maybe hydrogen abstraction) and homolytic cleavage of the Co−C bond. As long as the DMB-on form is stabilized by docking, heterolysis will be prevented because the Co−C bond is still quite stable.

Conclusion

The DFT in vacuo equilibrium structures of corrin models obtained here are believed to be the best data obtained to date by computational chemistry on cobalamin model systems, both because of the level of theory and the realistic model systems. We have studied the inherent in vacuo steric and electronic effects on the axial bonds in corrins.

The MeB_{12} model, **3**, had a Co−C bond length differing 0.02 Å from the crystal structure. Hence, this bond is well modeled. The axial Co−N bond was found to be 0.14 Å longer by DFT than in the crystal. DFT may overestimate this Co−N bond length, but it has no importance for the discussions of trends.

The DFT results support Dong et al., who found a cis-steric effect in cobalamins.[54] The elongation of the Co−C bond has been shown to have a large influence on the HOMO−LUMO gap (more strain gives a larger gap). This is mainly due to induced changes in the HOMO. These effects have minor influence on corrin folding. However, the effect of electron release into cobalt from the β-side elongates the trans Co−N_{ax} bond dramatically and in a quite systematic way. This is a theoretical verification of a combined β-side trans-electronic and trans-steric effect in corrins, the latter being more important. At the same time, more electron density on cobalt is found to stabilize both frontier orbitals of the corrins. The fact that the corrin ring is rather unaffected by the changes in the axial bonds seems to be a theoretical argument against a mechanochemical trigger mechanism. The histidine-on effect, which is basically the α-side steric and electronic effects, was only a 0.35 kcal/mol difference in HOMO−LUMO gaps of the two methylcorrins.

The equilibrium structures of MeB_{12} and $AdoB_{12}$ have been found to be somewhat different; notably we found a different electronic nature of the two Co−C bonds. According to B3LYP, the HOMO energy is higher in $AdoB_{12}$ than in MeB_{12}, favoring homolytic cleavage. This is due to the 5′-deoxyadenosyl group, which interacts with the corrin ring and induces more electron density on cobalt. This may explain part of the homolysis/heterolysis dichotomy. On the basis of the geometries obtained here and experimental results of other groups,[32−34] we suggest two very different reaction coordinates for the two coenzymes. In addition, the difference in HOMO and LUMO energies for the DFT structures of MeB_{12} (**3**) and $AdoB_{12}$ (**8**) models (Table 2) may explain the outcome of several photolysis experiments that give different outcomes in the heterolysis/homolysis competition.[55,56]

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