

THE NATURE OF THE Co-C BOND CLEAVAGE PROCESSES IN METHYLCOB(II)ALAMIN AND ADENOSYLCOB(III)ALAMIN

Tudor Spataru^{a*}, Francisco Fernandez^b

^aDepartment of Chemistry, Columbia University, New York, New York 10027, United States

^bNatural Sciences Department, Hostos Community College, 500 Grand Concourse, Bronx, New York 10451, United States

*email: ts2407@columbia.edu; phone: (1) -212-854-1080; fax: (1)-212-932-1289

In Memory of the Academician Constantin Turta

Abstract. Unfortunately, there are still significant disagreements between experimental and theoretical data of rate constants, energy barriers for Co-C bond cleavage process and coordination numbers of vitamin B₁₂ coenzyme species in spite of the remarkable efforts done by research community. Therefore, no grounded mechanisms for Co-C vitamin B₁₂ coenzyme bond breaking process and subsequent reactions have been found up to now. The influence of the mixing orbitals e.g. Pseudo-Jahn-Teller and similar effects on the reactions paths of bond-cleavage mechanisms of vitamin B₁₂ co-factors must be taken into account by utilizing multi-reference methods, in particular multi-configurational self-consistent field (MCSCF) method. Then, the change in total energy along the normal coordinate *Q* for the stretching mode including Co-C and Co-N bonds in vitamin B₁₂ cofactors is expected due to a “vibronic” coupling term, which couples an excited state and ground state by a second order derivative potential-energy operator. The strong state mixing effect is expected to lead to low energy barriers and to Co-C and Co-N axial bond cleavage events in agreement with experimental data. Afterward, the updated mechanisms of vitamin B₁₂ bio-processes can be determined.

Keywords: vitamin B₁₂, mechanism, bio-catalysis, Pseudo-Jahn-Teller effect, DFT, MCSCF.

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Introduction

Vitamin B₁₂ cofactor is one of eight B vitamins and is involved in mammalian cellular metabolism, influencing amino acid and DNA synthesis [1] and hematopoiesis. The best known human physiological function of vitamin B₁₂ is its role in promoting normal health in the brain and nervous system. Vitamin B₁₂ anemia can cause such neurologic dysfunction as weakness, fatigue, light-headedness, rapid heartbeat, rapid breathing, pale skin color, bruising, and bleeding (including bleeding gums). The more severe vitamin B₁₂ anemia dysfunctions are characterized by tingling or numbness of the fingers and toes, difficulty walking, mood changes, depression, memory loss, disorientation and, in most severe cases, dementia [2]. Vitamin B₁₂ deficiency has even been considered in playing a role in the development of Alzheimer’s disease [3-7].

The vitamin B₁₂ cofactor contains a cobalt atom surrounded by an equatorial corrin ring. Covalently linked to the central atom is a dimethylbenzimidazole that occupies one of the axial coordination positions. The opposite coordinate is available for several ligands in bio-medium or in solution; however, known biologically active structures containing adenosyl- or methyl have been considered most often (Figure 1).

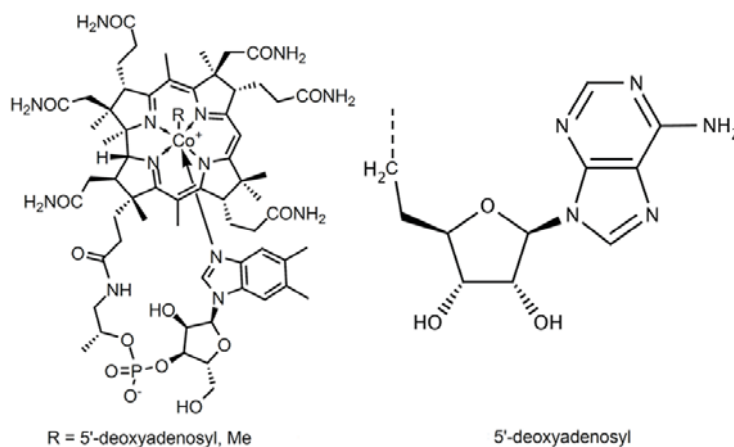


Figure 1. The structure of vitamin B₁₂ cofactor (left side) and of 5-deoxyadenosyl ligand (right side) with coordinating dash bond [8-10].

Vitamin B₁₂ is known to participate in at least two types of enzyme-catalyzed reactions [8-10] in the human body: a) isomerase rearrangements with participation of the adenosylcobalamin form of vitamin B₁₂; b) methyltransferases with participation of the methylcobalamin form of vitamin B₁₂. To date, human consumption of the various vitamin B₁₂ varieties and their mechanisms of action in the human body are still under study. The best form of vitamin B₁₂ to be recommended to patients is still not fully established in the medical community [6-10]. Moreover, the recommended dosage of vitamin B₁₂ varies from one author to another [3-7]. One of the more controversial areas of research regarding the vitamin B₁₂ mechanism is its activation that starts with Co-C bond cleavage under influence of electron transfer and/or substrate influence. There is little agreement between experimental and theoretical data in spite of a significant amount of research on this problem. The mechanism of vitamin B₁₂ must be updated with correct theoretical and experimental data that is in agreement with each other.

This review is dedicated to a better understanding of the chemistry involved with B₁₂ bio-catalysis in searching of a firmer foundation upon which we can treat the various forms of anemia and neurologic dysfunction diseases associated with vitamin B₁₂.

The mechanisms of methylcobalamin and adenosylcobalamin activation

Vitamin B₁₂ and its derivatives have received considerable attention for their use in organic chemistry [11-19]. The cobalamin cofactor plays an integral role in methyl transfer by donating methyl groups to homocysteine and accepting them from CH₃-H₄ folate [20-22]. The biological reactions catalyzed by cobalamin-dependent methionine synthase generally include as a primary turnover the enzyme-bound cobalamin prosthetic group cycling between methylcob(III)alamin and cob(I)alamin forms. All corrinoid-dependent methyl transferases bind the corrinoid cofactor with displacement of the dimethylbenzimidazole ligand: in most cases the side chain of histidine residue (Figure 2) from the enzyme is a new ligand [11,23-28].

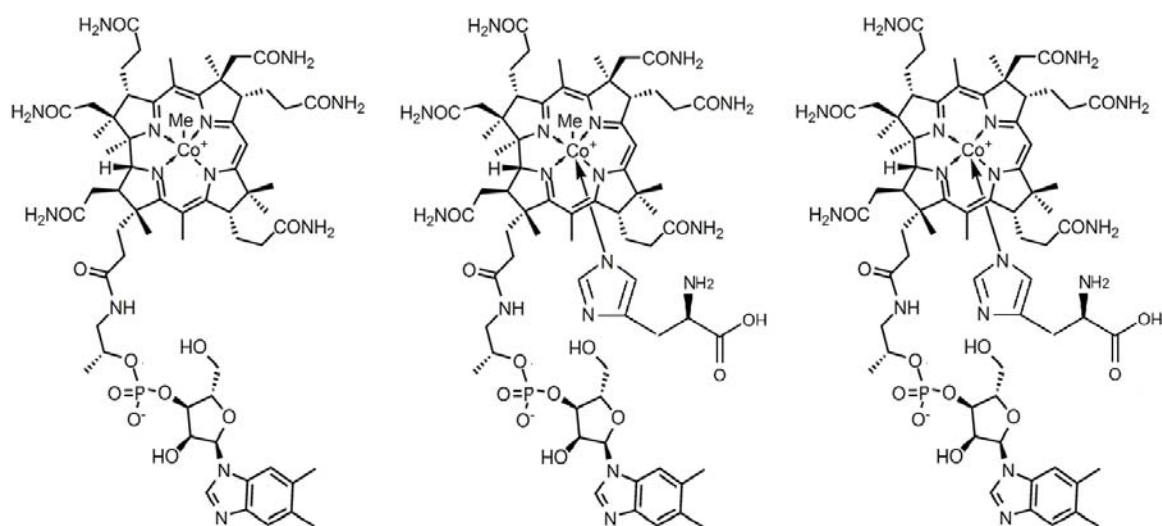


Figure 2. The schemes of the methylcobalamin coenzyme common structures found by X-ray diffractions [11,23-28].

Adenosylcobalamin is the cofactor of several enzymatic processes [29-37], such as melanoma cell adhesion molecule (MCAM), glutamate mutase, methyleneglutaratemutase, class II ribonucleoside triphosphate reductase, ethanolamine ammonia lysase, and diol- glycerol dehydratase. A common feature of these enzymes is that the Co-C bond of adenosylcobalamin is cleaved homolytically to initiate the reaction, giving rise to a five-coordinate cob(II)alamin and an adenosyl radical. Measurements of the rate constant for cob(II)alamin formation demonstrate that the enzyme increases the rate of homolysis of the Co-C bond by 10¹²-fold both for B₁₂ – dependent methylmalonyl-Co-A mutase and adenosylcobalamin-dependent glutamate mutases [33,34]. Finally, we should point out the differences between the initial stages in methyl- and adenosyl-dependent mutases considering the Co-C cleavage process. As it is depicted in Figure 3: (a) the methylcobalamin-dependent methionine synthase generally includes as a primary turnover the cycling between Co(III) methylcobalamin and cob(I)alamin, which allows the heterolytic Co-C cleavage process, while in the adenosyl-cobalamin-dependent mutases the Co-C bond of adenosylcobalamin is cleaved homolytically, giving rise to a five-coordinate cob(II)alamin and an adenosyl radical; (b) the association of the substrate and adenosyl-ligand during the Co-C cleavage process in adenosylcobalamin dependent mutases has been proved by various experimental data including X-ray diffraction results [35-37], while such effect is totally absent in studies regarding methylcobalamin-dependent mutases.

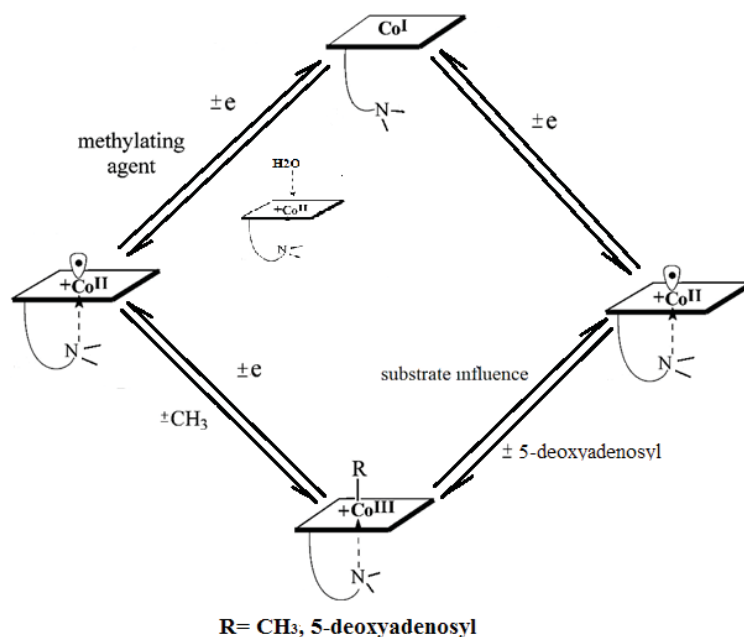


Figure 3. The generalized scheme of the methylcobalamin and adenosylcobalamin coenzyme turnovers [38-67].

The Co-C cleavage and electron transfer processes have been studied by different methods such as electrochemistry [38-40], thermo- [41] or photochemistry [42-46] of methylcobalamin Co(III) by a significant number of researchers. As a result, many triggering factors of Co-C bond breaking have been considered and various mechanistic mechanisms of the Co-C cleavage process have been proposed. Many researchers have considered axial ligand trans-influence, steric effects, structural strain, protein influence, in addition to others [47-58] as determinant factors in the Co-C cleavage process. The hydrogen transfer from the substrate to 5-deoxysenosyl ligand has also been considered as a triggering factor of the Co-C bond breaking [59-67]. These suggestions have been largely unconfirmed by subsequent data. Only substrate influence on the Co-C cleavage process in the case of the adenosylcobalamin vitamin B₁₂ species has been confirmed [35-37]. New approaches and new strategies are needed for the analysis of this challenging biological phenomenon.

The reductive activation mechanism of methylcobalamin has been studied by D. Lexa and J.M. Sav ant using cyclic voltammetry of two similar compounds, methylcobalamine (base-on species) and methylcobalamide (base-off species) in DMF-1-propanol [38]. The methylcobalamide compound shows a single irreversible cathodic wave at low sweep rates corresponding to the reductive cleavage of the cobalt-carbon bond. At the slow scan rate of 0.3 V/s the cyclic voltammetry (CV) of methylcobalamin showed no return wave; however, as the scan rate increased to 10 V/s and above at -20  C, a return wave appeared which allowed Lexa and Sav ant [38] to propose the multistep electrochemical mechanism (Figure 4). Upon raising the sweep rate the cathodic wave becomes progressively reversible, clearly showing the existence of a one-electron intermediate before cleavage of the cobalt-carbon bond.

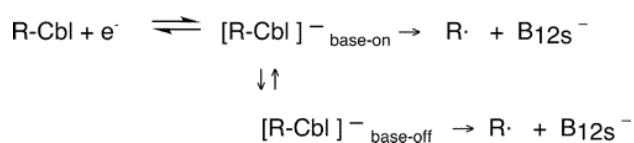


Figure 4. The scheme of the methylcobalamin reductive electrochemical mechanism [38,39].

Here, R is a methyl group.

We [39,40] and Martin & Finke [41] have determined similar methylcobalamine reductive mechanisms.

Unfortunately, the above mechanism is not fully supported by theory when considering DFT calculation data [39,40]. Additionally, the generally accepted methylcobalamin reductive mechanism (Figures 2 and 3), based on various experimental methods including X-ray diffraction, does not fit exactly with the electrochemistry based methylcobalamin reductive mechanism (Figure 4). It is obvious that experimental and theoretical data do not combine to provide a clear understanding of vitamin B₁₂ activation in the human body. Deeper and more detailed studies using adequate and modern methods should be performed to ensure a sound basis of the mechanism of activation of vitamin B₁₂.

Density Functional Theory (DFT) and Quantum Mechanics/ Molecular Mechanics (QM/MM) calculations on vitamin B₁₂ cofactors

We [39,40] and others have performed DFT calculations of vitamin B₁₂ models [68-100]. Consequently, various properties have been studied, including electron densities and reduction potentials determination [69,76,88,91,96], the comparison of DFT and CASSCF electronic structure data of truncated models of methylcobalamin species [70,71,74,81,85], the influence of various factors on the Co-C bond forming and cleavage in methyl- and 5-adenosylcobalamin models [68,72-76,79,80,83,84,89,90,92,94,97,99,100], and excited states, spectroscopy and photo-dissociation analysis of vitamin B₁₂ species [78,82,86,87,93,95,98]. We used DFT calculations on Me-Cbl models to calculate total energy as a function of Co-C bond distance as the bond is stretched [39]. The generally used base-on model of R-Cbl(III)(R=CH₃) includes the full corrin ring with all side-chain groups replaced by hydrogen atoms and with the dimethylbenzimidazole base ligand replaced by benzimidazole (or imidazole), as it is shown in Figure 5.

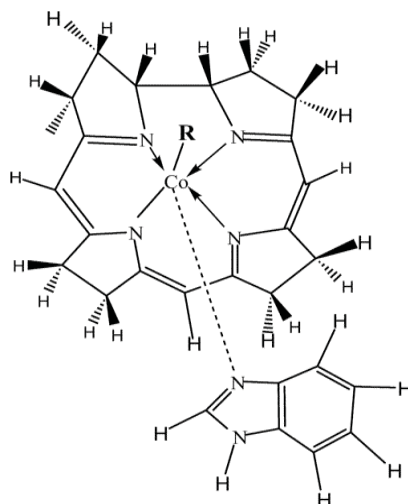


Figure 5. The methylcobalamin model used in DFT calculations [39, 106].

This approach was taken for construction of base-on and base-off models of positively charged CH₃-Co(III)⁺ and likewise for the neutral reduced CH₃-Co(II) [41]. The optimized DFT geometry for all four species at B3LYP/LANL2DZ theory level showed nearly the same equilibrium C-Co bond distance of around 2.00Å. The dissociation total energy barrier heights from single-point calculations for both the base-on and base-off CH₃-Co(III)⁺ models were almost the same, ca. 2.8 eV (Figure 6); however, for the base-on CH₃-Co(II) model, this dissociation energy was much lower, ca. 1.6 eV, and for the base-off species, even lower, ca. 1.1 eV.

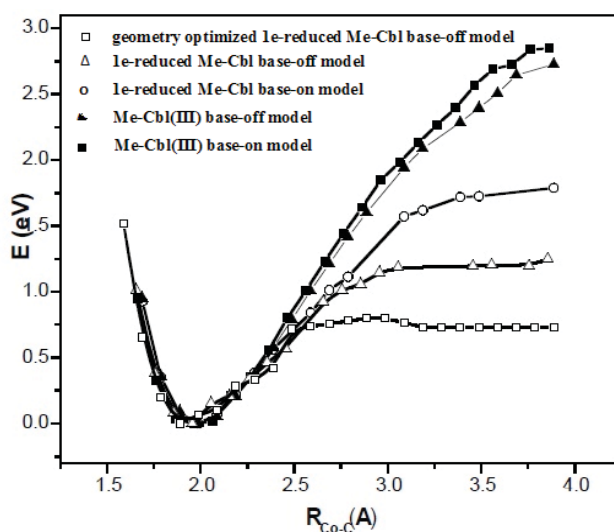


Figure 6. Total B3LYP/LANL2DZ energy (eV) as a function of Co-C bond distance (Å) for methylcobalamin model compounds for different redox species and axial base conditions. The energy of all curves has been aligned at the minimum [39,106].

Despite the progress made by the research community on the theoretical explanation of vitamin B₁₂ cofactor reaction, there are still significant disagreements of theoretical results with experimental data. Thus, our calculation of the total energy with geometry optimization at each constrained C-Co bond length for the base-off CH₃-Co(II) model gives the lowest energy barrier for C-Co bond breaking (value of 0.7 eV, Figure 6) [39,106]. However, even with this barrier, a kinetic calculation shows that fast bond cleavage is improbable. Furthermore, the energy barrier of the Co-C bond breaking is much higher for base-on compared to the base-off species [39,68,106]. Our estimations show that the energy barrier of the geometry optimized 1e-reduced base-on specie is higher by about 0.5 eV compared to the energy barrier of the geometry optimized 1e-reduced base-off specie (see Figure 7 for single points calculations). Therefore, according to DFT calculations the rate constant of Co-C bond cleavage has a much lower value for base-on species compared to the rate constant of similar reactions for base-off species. This enters a flagrant contradiction with the experimental data, which demonstrates that the reaction rate constant of Co-C bond breaking is much higher in base-on species compared with the same reaction rate constant for base-off species [38]. Moreover, base-off methylcob(II)alamin compound cannot be considered in Co-C bond cleavage reaction of bio-reactions since is predicting a four coordinate vitamin B₁₂ specie immediately after a Co-C bond cleavage of methylcobalamin compound [39,68,106]. Instead, several experimental results show that methylco(II)calamine cofactor is five coordinate [23-28,101,102] immediately after Co-C bond cleavage.

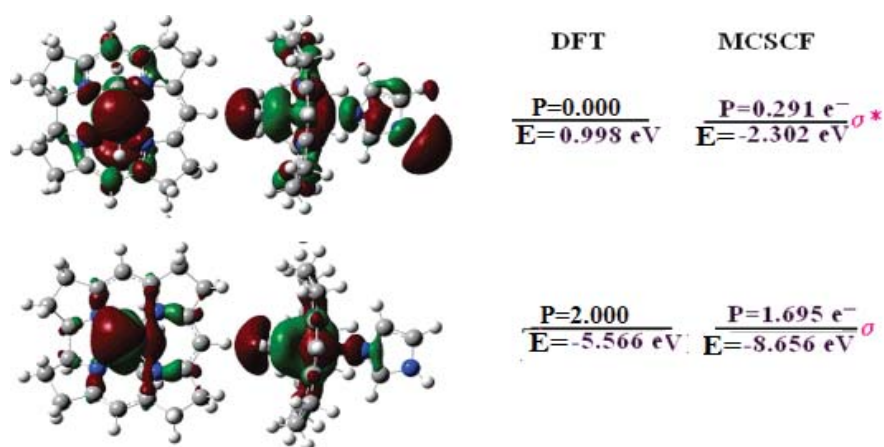


Figure 7. The methylco(II)balamin DFT-B3LYP/LANDL2DZ and MCSCF (9,7) (CASSCF) surfaces, energies and populations of σ and σ^* MO [39,106].

Another unfortunate discrepancy is that theoretical results support the existence of a six-coordinate methylcobal(II)aminecofactor with dimethylbenzimidazol ligand bonded to cobalt ion. Such a six-coordinate specie, which has not been mentioned by any experiment up to now, has a clear minimum (Figure 6) of total energy in the DFT method [39,106], testifying in favor of its stability. Finally, no theoretical model gave the expected Co-C bond cleavage energy barrier for the methylcob(II)alamin models. The closest to the desired results up to now have been obtained by using QM/MM and DFT methods, which, unfortunately, still give greater than expected barriers for either, base-on or base-off species, equal to 8.3 kcal/mol, 10.5 kcal/mol, and 7.3 kcal/mol, respectively [72,73,80] (in *in vivo* Co-C cleavage process is supposed to run without barrier). Finally, we conclude that there is an evident requirement to improve the models used in theoretical calculations and the use of more advanced theoretical treatments for the study of vitamin B₁₂ activation.

The multi-configuration effects in methylcob(II)alamin and its Co-C bond cleavage

Although, the study of the *in-situ* electron transfer in reduced cobalamin compounds is quite a challenging problem, the real catalytic process and electronic transfer in cobalamin-dependent enzymes are even more complicated. For instance, the electron transfer problem includes also the role of mixed valence ferredoxin compounds, which participate in electron transfer [103-105] to a series of biosystems and participate into turn-over of cobalamin-dependent enzyme processes. The mixed valence ferredoxin compounds are a common example of vibronic (Jahn-Teller) systems in chemistry, due to their symmetry. On the other hand, our recent electron structure studies show that the HOMO-LUMO and Co-C σ - σ^* MO gaps are significantly smaller in the Co(II) methylcobalamin system compared with Co(III) methylcobalamin, proving that the orbital mixing is effective [106]. Therefore, the activation of Co-C bond cleavage in cobalamin bio-chemistry must be treated as an orbital mixing process and the Co(II) methylcobalamin system cannot be treated correctly by DFT methods [107], as it is a Pseudo-Jahn-Teller system [108]. Such orbital mixing leads to

a fractional population of the σ and σ^* orbitals, including also several bonding occupied and antibonding unoccupied orbitals [106]. The σ^* orbital, which is situated significantly above π^* SOMO, is coupled strongly with formal σ . As a result, the energy of these two σ and σ^* molecular orbitals change significantly during the geometry optimization (Figure 7). The population of the σ^* antibonding orbital increase and the population of the σ bonding orbital decrease, thus “weakening” the force constants along the Co-C and Co-N bonds. This strong state mixing (similar to the pseudo-Jahn-Teller effect) leads to an increase in the Co-C bond and Co-N_{ax} bond lengths with about 0.2 Å for base-off Co(II) methylcobalamin model compounds and base-on Co(II) methylcobalamin model compounds (Figure 8). This coupling explains also the much lower Co-C bond dissociation enthalpy and much faster bond cleavage rate for the one-electron reduced methylcobalamin radical anion compared to the methylcobalamin neutral system. Such calculations like MCSCF with strong orbital coupling are extremely sensitive to any perturbation. We have obtained a similar result for Co-C bond cleavage in the case of the Co(III) adenosylcobalamin system under the perturbation (slight interaction influence) of the enzyme substrate. Therefore, it is reasonable to consider the influence of the substrate as a perturbation factor of the electronic structure influencing the Co-C and Co-N bond breaking for vitamin B₁₂ cofactors species. We believe that this concept can be generalized for electron transfer or for substrate influence and subsequent bond cleavage in important biological systems.

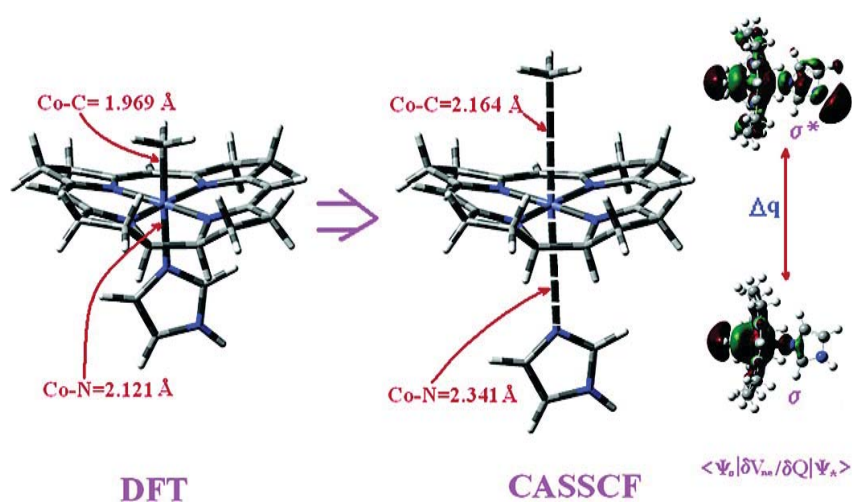


Figure 8. The cartoon of σ and σ^* MO coupling influence on axial Co-C and Co-N bonds of one electron reduced methylcobalamin [39,106].

Generally speaking, the MCSCF method, which takes into account multi-configuration interactions, is among the most accurate methods of electronic structure calculations. Unfortunately, the number of electrons and orbitals taken into consideration in our previous calculation [106] are not in harmony with the needs for totally accurate calculation results of the MCSCF method with a complete active space that can be compared with experimental data and that can serve as the basis to develop a precise and reliable mechanism of activation for vitamin B₁₂. In the computing process it is necessary to take into account more orbitals (including a complete MCSCF active space) and a larger truer-to-size model of vitamin B₁₂ should be used in the calculation according to the needs of today’s knowledge of the mechanism of activation.

Conclusions

There are several possible mechanisms for the intramolecular electron transfer step, including distortion and coupling of orbitals of different symmetries, the possibility of a conical intersection, and a vibronic coupling mechanism—all of which are not mutually exclusive. The bond dissociation energy (BDE) for the cobalamin base-off species gives a value of 0.7 eV or ~16 kcal/mol based on a π^* ground state potential energy dissociation curve calculated at the B3LYP/LANL2DZ level and optimized at each Co-C distance; however, this result is considered inaccurate because it is still too large to explain the experimental electrochemical kinetics.

The mechanism describing the bond-breaking of the base-off species in the solvent cage cannot explain the large difference in cleavage rates between reduced MeCbl and MeCbl⁺, and DFT calculations therefore give inaccurate energy barriers in this situation. The alternative methodologies such as open-shell multi-configurational methods in theoretical calculations and perturbation factors along with the bond length estimations and mechanism fitting must be used for studying these processes.

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