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THE Co-N BOND CLEAVAGE IN THE ADENOSYNCOBALAMIN COFACTOR IN ADVANCE TO GLUTAMATE MUTASE AND METHYLMALONYL-CoA MUTASE PROCESSES

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Abstract. The *in vivo* experiments show that the adenosylcobalamin cofactor in glutamate mutase and methylmalonyl-CoA mutase processes lose its dimethylbenzimidazole axial ligand before starting the enzymatic processes. Complete active space self-consistent field geometry optimization of the vitamin B12 active forms plus substrates joint models have been performed. These joint models include the adenosylcobalamin cofactor, the carboxyl negative ion model of the studied processes' active substrates, and the histidine molecule. Partial electronic density is transferred from the highest occupied substrate molecular orbitals to the lowest unoccupied antibonding molecular orbitals, which consist of corrin ring and dimethylbenzimidazole ligand common molecular orbitals during the multi-configurational self-consistent field molecular orbital mixing process. As a result, the Co-N axial bond is permanently elongated during the complete active space self-consistent field geometry optimization until its complete rupture and until the removal of the dimethylbenzimidazole ligand from the central cobalt atom and the corrin ring is complete. The Co-N bond cleavage in the adenosylcobalamin cofactors in the studied processes is running as no energy barrier process under the influence of their active substrates and histidine molecule.

Keywords: glutamate mutase, methylmalonyl-CoA mutase, adenosylcobalamin cofactor, vitamin B12, Pseudo-Jahn-Teller effect.

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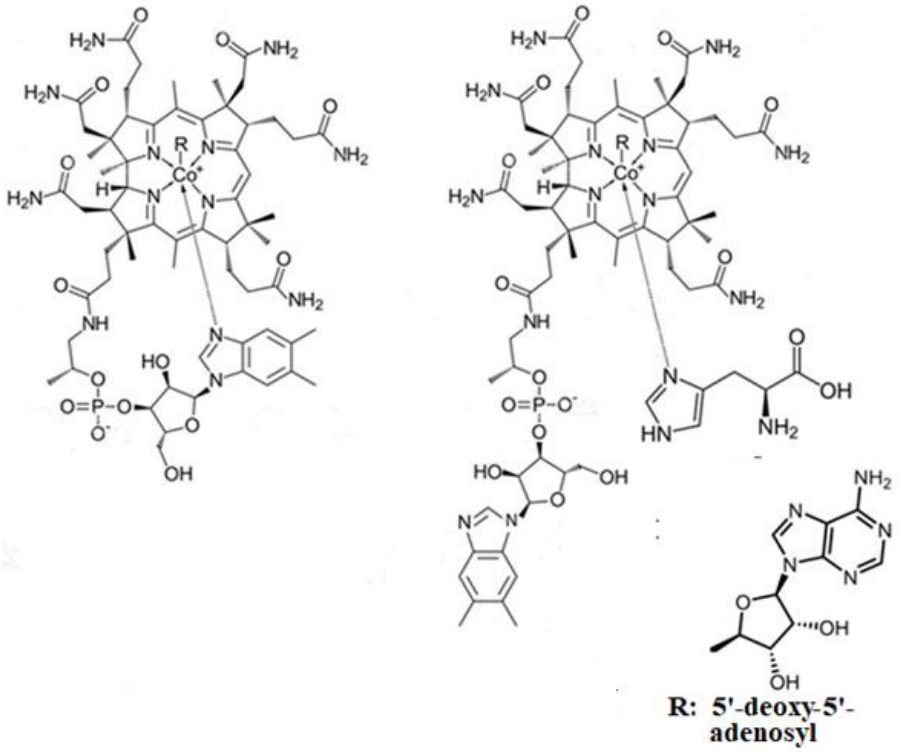
Introduction

Vitamin B12 deficiency in the human body causes several diseases and dysfunctions such as decreased red blood cell level, decreased ability to understand daily activities, impaired movement, genetic disorder, intestinal inflammation, and even death in case of major deficiencies [1-5]. Adenosylcobalamin, one of the B12 cofactors, consists of the corrin ring, side chains, axial ligands, and Co central atom. Its structure is well known and is shown in Figure 1. The upper axial ligand is 5'-deoxy-5'-adenosyl radical, while dimethylbenzimidazole is a second axial ligand in regular adenosylcobalamin cofactor, although structural studies have shown that it is substituted by histidine during the glutamate mutase and methylmalonyl-CoA mutase processes (Figure 1) [6,7]. Adenosylcobalamin cofactor catalyzes the skeleton mutase glutamate mutase and methylmalonyl-CoA mutase bioprocesses (Scheme 1).

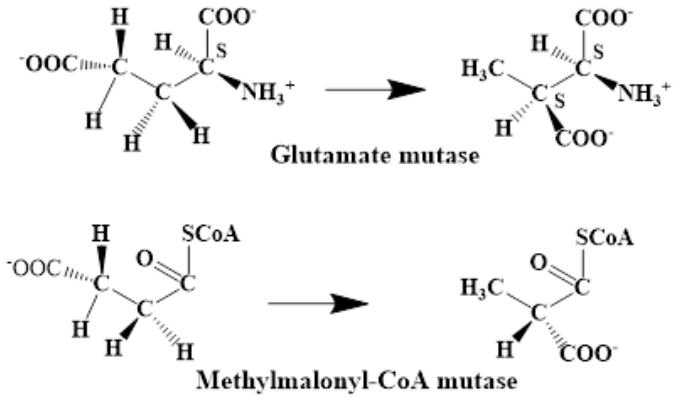
Adenosylcobalamin cofactor-dependent processes have been studied experimentally and theoretically. Particularly, X-ray studies have shown that the dimethylbenzimidazole ligand is replaced *in vivo* with a histidine molecule [6,7] in the adenosylcobalamin cofactor in advance of the glutamate mutase and methylmalonyl-CoA mutase processes the general analysis allowed to postulate the adenosylcobalamin-dependent processes general mechanisms [8-14], while kinetic, spectroscopic and electronic structure studies have evidenced various particularities of the Co-C or Co-N axial bond cleavage process in the adenosylcobalamin cofactor during the adenosylcobalamin-dependent processes on the basis of the experimental [15-23] and theoretical pieces of evidence [24-35]. Among the theoretical study methods, the most accessible from the point of view of the used computer resources economy are the density functional theory (DFT) and quantum mechanics/molecular mechanics (QM/MM) methods, based on the DFT methodology. Therefore, DFT and QM/MM based on DFT theory have been used to study the mechanism of the adenosylcobalamin

cofactor-dependent processes [24-32]. The applications of these methods to the study of the Co-C and Co-N cleavage bonds in adenosylcobalamin cofactor generally result in significant barriers [24-32], in flagrant contradiction with the experimental data, which show a close to the unity equilibrium constant for Co-C bond cleavage reaction (see [17,30] and references therein). Assumptions have been made that would diminish the energy barrier of these reactions. One of them is the cofactor adenosylcobalamin's electron reduction before the catalytic process occurs [25]. However, all experimental data [8-23] show that the catalytic process starts with the non-reduced adenosylcobalamin cofactor particles. Moreover, the energy barrier of the Co-C bond cleavage reaction obtained in these calculations is still significant [25]. Another assumption that has been made is the compensation of the energy barrier of the Co-C bond cleavage reaction by the gain in energy in the substrate processes [30]. Although the substrates were calculated using the MM method [30], the energy of which is not commensurable with the DFT energy due to the different theoretical backgrounds.



(a) *(b)*

Figure 1. The adenosylcobalamin base-on forms: the regular base-on form *(a)* and base-on form with the dimethylbenzimidazole ligand substituted by histidine molecule *(b)*.



Scheme 1. The skeleton glutamate mutase and methylmalonyl-CoA mutase bioprocesses catalyzed by adenosylcobalamin cofactor.

Finally, the relationship between the Co-C bond cleavage and the hydrogen transfer from the substrate to the 5'-deoxy-5'-adenosyl radical reactions, which occur in these processes, has not been undoubtedly resolved [24-32] by using the DFT or QM/MM method, based on DFT theory. These inconveniences are explained by the fact that the molecular orbital mixing, *e.g.*, the Pseudo-Jahn-Teller-Effect contribution, is crucial for these mechanisms' reaction behavior [32,35], which correct determination is beyond the limits of the DFT-based methods, as proved by Bersuker, I.B. [36].

The mechanisms of the adenosylcobalamin cofactor-dependent bio-processes have been theoretically recently resolved [32]. The multi-configurational self-consistent field (MCSCF) was a key method for a detailed electronic structure and mechanism of the adenosylcobalamin cofactor-dependent processes determinations since the Pseudo-Jahn-Teller-Effect is taken into account by the MCSCF methods. The MCSCF geometry optimization process shows that during adenosylcobalamin cofactor-dependent bioprocesses, the Co-C bond cleaves, releasing a Co^{2+} cobalamin species and the 5'-deoxy-5'-adenosyl radical. The MCSCF electron structure calculations show that an initial electron density transfer occurs from the substrate to the adenosylcob(III)alamin cofactor, under the influence of which the Co-C bond is cleaved [32]. Further MCSCF electronic structure calculations show that the 5'-deoxy-5'-adenosyl radical extracts hydrogen from the substrate [32], testifying in favor of the tunneling and Pseudo-Jahn-Teller-Effect nature of the hydrogen transfer. All reactions in the studied processes occur in the absence of an energy barrier [32]. To date, in theoretical calculations carried out for the study of glutamate mutase and methylmalonyl-CoA mutase processes, a structural model in which imidazole ligand, modeling the histidine molecule, which substituted the dimethylbenzimidazole ligand in adenosylcobalamin cofactor, is used [24-32]. To substitute the dimethylbenzimidazole ligand with a histidine molecule, the first one must be removed from the central cobalt atom by breaking the Co-N axial bond. The aim of this study is to determine the mechanism of the Co-N bond cleavage in the adenosylcobalamin cofactor *in vivo* under the influence of the substrates present in the bio-cavities of glutamate mutase and methylmalonyl-CoA mutase processes.

Computational details

One can observe that the substrates of both glutamate mutase and methylmalonyl-CoA mutase bioprocesses include the $–COO^-$ carboxyl group (Scheme 1) and can, therefore, be modeled by the CH_3COO^- structure. The geometry optimization of the glutamate mutase and methylmalonyl-CoA mutase bioprocesses' initial step model, which includes the adenosylcobalamin cofactor and substrates joint model (Figure 2) has been performed by using the complete active space self-consistent field (CASSCF) method. The calculated models' total charge and spin are equal to 0 and 1, respectively. 12 orbitals and 12 electrons have been taken into CASSCF active space for studied adenosylcobalamin-dependent bioprocesses. The increase of the CASSCF active area to (14,14) does not lead to modifying electronic structure results for the studied systems. Further increase of the active area of such large systems using the NwChem code is not possible, in principle, due to the computing capabilities of the supercomputers on the university campus. A 6-31G** basis set for the cobalt and oxygen atoms and a 6-31G basis set for the remainder of the atoms have been used for all geometry optimization procedures. Such a basis set was used successfully for an electronic structure of the cofactor-dependent bio-process calculations [32,33].

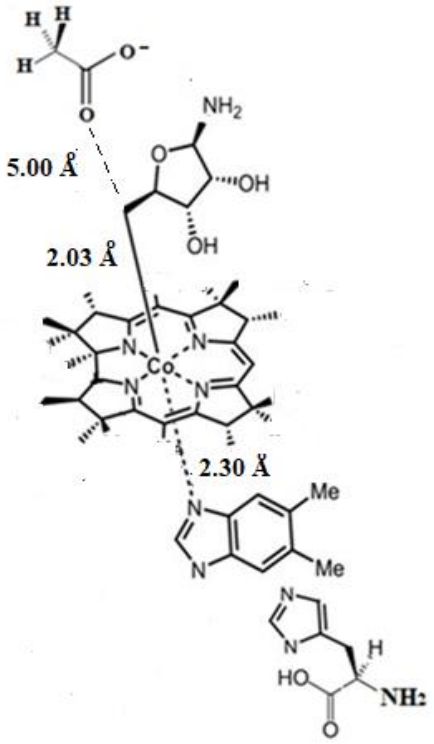


Figure 2. Structure of the CASSCF electronic structure calculation model.

NwChem computational software [38] was the main code used to optimize the geometry of the CASSCF calculated model. A quasi-newton optimization with line searches and approximate energy Hessian updates has been used as the optimization procedure. The side chains of the adenosylcobalamin cofactors and the outside structure of the 5'-adenosyl-axial-ligand structure have been replaced by hydrogen atoms (Figure 2). The calculations should include dynamic factors for a more severe CASPT2 approximation. However, the results of these calculations strongly agree with the experimental evidence, and this gives confidence that they correctly enough reflect the nature of the studied processes.

Results and discussion

The most likely factor that would cause the cleavage of the Co-N axial bond, *i.e.*, breaking the chemical bond between the dimethylbenzimidazole ligand and the central cobalt atom, is the influence of substrates on the adenosylcobalamin cofactor. As shown in Scheme 1, the COO^--active group is the common feature of the glutamate mutase and methylmalonyl-CoA mutase process substrates. On the other hand, according to X-ray data [6-9], there are several residues in the proximity of the axial dimethylbenzimidazole ligand, such as histidine, glycine, tyrosine, arginine, threonine, serine, lysine, glutamine, leucine, asparagine, and tartrate, which can influence the behavior of the adenosylcobalamin cofactor.

It has been shown that vitamin B12-dependent enzyme processes occur in the absence of total energy barriers [32,33]. Therefore, it was intended to find models in which the preliminary step of these processes also takes place in the absence of the total energy barrier. In all models in which, in addition to the adenosylcobalamin cofactor's structure, one, two, or three substrates presented above have been added. The CASSCF geometry optimization of these joint models showed that the cleavage of the Co-N bond, in most cases, occurs with energy barriers except for the model presented in Figure 2.

The adenosylcobalamin cofactor catalyzes a series of processes in which the structure and composition of several substrates are modified. As shown above, in glutamate mutase and in methylmalonyl-CoA mutase processes, the dimethylbenzimidazole ligand is replaced by the histidine molecule [6,7]. Therefore, to participate in some of its catalytic reactions, the adenosylcobalamin cofactor molecule must release the dimethylbenzimidazole ligand to be able to bind histidine to the cobalt central atom. As stated above, the Co-N axial bond cleavage most probably takes place under the influence of the substrates present in the cavity of this vitamin B12 cofactor.

Among the most present structures in the studied adenosylcobalamin cofactor cavity are the substrates that are subjected to the catalytic activity of the glutamate mutase and methylmalonyl-CoA mutase processes from one side and the histidine molecule from another side. As shown above, the active substrates of the glutamate mutase and methylmalonyl-CoA mutase processes can be modeled by the CH_3COO^- formula. Thus, CASSCF geometry optimization models of the preliminary step of the adenosylcobalamin-dependent processes include adenosylcobalamin cofactor molecule, histidine molecule, and CH_3COO^- structure (Figure 2). The glutamate mutase and methylmalonyl-CoA mutase processes preliminary step model (Figure 2) CASSCF geometry optimization was started with the Co-C axial bond distance equal to 2.03 Å and with the Co-N axial bond distance equal to 2.30 Å [6,7,39]. In the MCSCF calculation method, there are formally no highest occupied molecular orbitals (HOMO) and lowest unoccupied molecular orbitals (LUMO) due to orbitals mixing and fractional electronic density orbitals populations. However, to avoid long and cumbersome explanations, we will name HOMO the highest-positioned molecular orbital with an electronic population greater than one electron and LUMO the lowest orbital with less than one electron density population. The names of the other orbitals are made accordingly. And the differences from zero electronic populations of the LUMOs orbitals, influencing the initial structure stability of the calculated compounds, will consider the consequence of the Pseudo-Jahn-Teller-Effect [40], in which such partial electron density populations are the result of the charge transfers from the HOMOs to LUMOs. At the beginning of the MCSCF geometry optimization of the studied adenosylcobalamin-dependent processes' preliminary step model (Figure 2), the electron density transfer from the highest occupied molecular orbitals to the lowest unoccupied molecular orbitals takes place in the process of molecular orbitals mixing. It should be noted that only the electronic density transfers from three HOMOs to three LUMOs of each calculated system are quite significant and can influence the calculated model's CASSCF geometry

optimization procedure pathway. The other HOMOs in the active zone of the CASSCF calculations represent a combination of atomic orbitals of the corrin ring with those of the Co-C bond atoms, and LUMOs a combination of atomic orbitals of the corrin ring and of the Co-N bond atoms, both for calculations with the active areas (12,12) and (14,14). The population of each HOMOs with approximately two electrons and of each LUMOs with almost zero electrons does not allow to consider them as factors that would visibly influence the studied processes. Interestingly, HOMOs/LUMOs shapes do not observably change, and all the electronic density populations of HOMO1, HOMO2, and HOMO3, which in sum is equal to approximately 5.73 e^-, for the model in Figure 3, and the electronic density populations of LUMO1, LUMO2, and LUMO3, which in sum is equal to 0.27 e^- remain unchanged throughout the whole CASSCF geometry optimization procedure of the calculated models.

It can be seen from Figure 3 that HOMO1, HOMO2, and HOMO3 are substrate molecular orbitals, while LUMO1, LUMO2, and LUMO3 are antibonding orbitals, which include the atomic orbitals of the corrin ring and of the dimethylbenzimidazole ligand. The CASSCF geometry optimization leads to the permanent Co-N bond distance increasing up to more than 4.00 Å distance, where there is no cobalt-axial nitrogen or corrin ring-axial nitrogen interaction. It turns out that the HOMO-LUMO electron density transfer creates a repulsive force of the dimethylbenzimidazole ligand from the cobalt central atom and from the corrin ring of the adenosylcobalamin cofactor molecule. On the other hand, the Co-N axial bond is the weakest coordination bond in vitamin B12 cofactors, its length being between 2.30 Å and 2.50 Å in biological materials and 2.24 Å in non-biological materials [6,7,39]. The repulsive force between the dimethylbenzimidazole ligand and the central cobalt atom (and the corrin ring) increases the distance of the Co-N chemical bond until its complete cleavage. Thus, the mixing of orbitals and charge transfer in the electronic structure of the calculated CASSCF model leads to the complete rupture of the Co-N bond in the CASSCF geometry optimization process and to the removal of the dimethylbenzimidazole ligand from the central atom and the corrin ring (Figure 4). The whole process of the Co-N bond cleavage is running in the absence of the total energy barrier. This result fits the existing X-ray data [6,7].

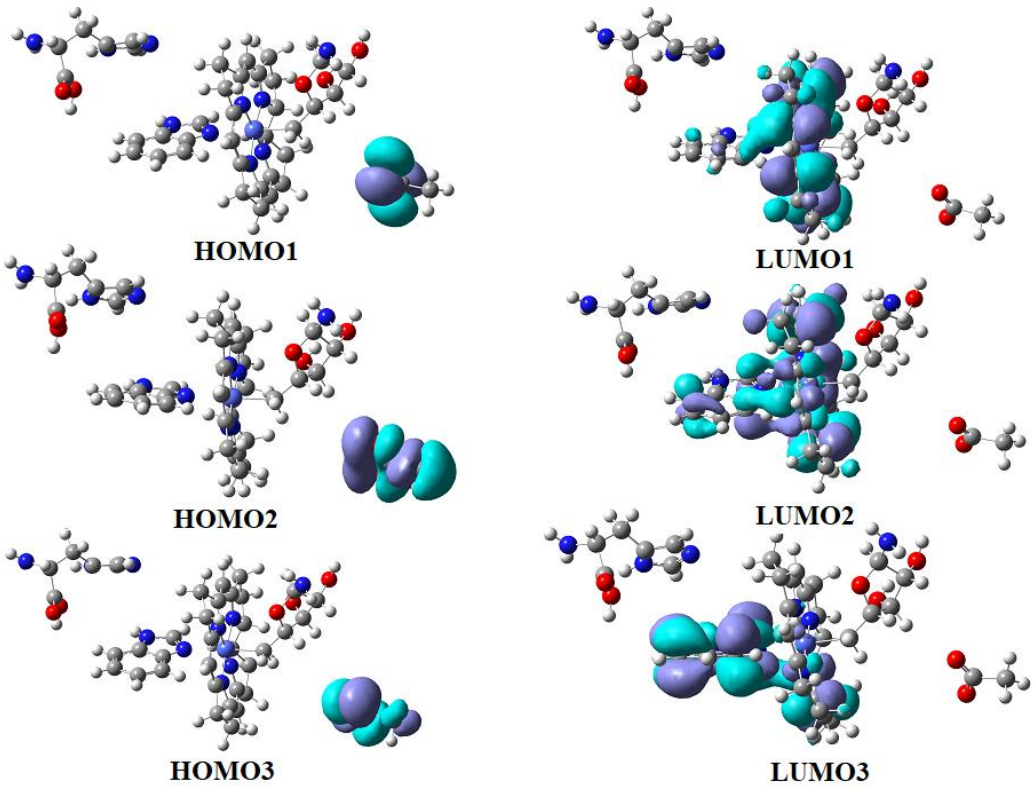
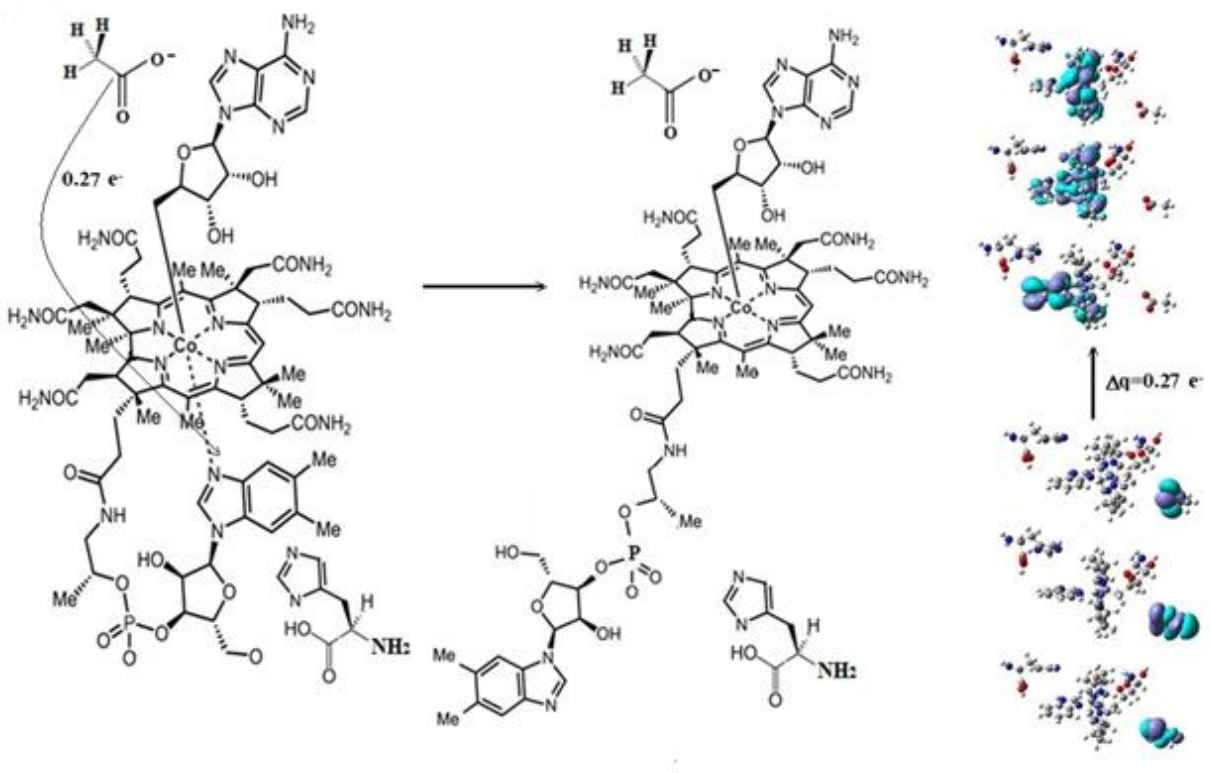


Figure 3. The MCSCF frontier molecular orbital surfaces of the histidine, adenosylcobalamin, and CH_3COO^- joint model (Figure 2, see the text).

It should be mentioned that the histidine molecule is situated at a distance of approximately 4.00 Å from the dimethylbenzimidazole ligand during the CASSCF geometry optimization process. In principle, at this distance, there are no regular chemical interactions. Nevertheless, both structures move simultaneously and equally away from the corrin ring and cobalt atom, keeping the distance between histidine and dimethylbenzimidazole ligands unchanged even if no constraints were applied during the CASSCF geometry optimization. But starting with a Co-N bond distance equal to approximately 4.00 Å, the histidine molecule and the dimethylbenzimidazole ligand distance themselves from each other so that they become independent entities that stop moving simultaneously and together in their previously formed block. That is, starting at the Co-N bond distance of about 4.00 Å and more, during the CASSCF geometry optimization procedure, the histidine molecule and the dimethylbenzimidazole ligand become structures that no longer interact. In this situation, the dimethylbenzimidazole ligand no longer depends on a possible interaction with the histidine molecule and moves independently, interacting with various substrates from the biochemical environment, possibly moving completely away from the central cobalt atom and corrin ring, or becoming the ligand, which tends to approach the central cobalt atom of the adenosylcobalamin cofactor and form the Co-N chemical bond. Likewise, the histidine molecule can make a separate movement and be located in the enzymatic environment of the adenosylcobalamin cofactor or bond its central cobalt atom and become an axial ligand. The DFT and CASSCF geometry optimizations show that the formation of the Co-N bond takes place in the absence of the total energy barrier due to the fact that in the formed base-off adenosylcobalamin species, the oxidation state of the cobalt central atom is of +3 oxidation state. Therefore, the formation of the Co-N chemical bond with the dimethylbenzimidazole ligand or with the histidine molecule depends on the relative energetic relationship between the two states in which one of the possible ligands of the cobalt atom is located far from the cobalt atom and another is in chemical connection with it forming the axial Co-N bond.



(a) *(b)*

Figure 4. The mechanism of the Co-N bond cleavage in the adenosylcobalamin cofactor under the influence of the histidine and CH_3COO^- substrates: substrate-adenosylcobalamin charge transfer *(a)*; the cleavage of the Co-N bond *(b)*.

The energetic analysis of the formation of these two states requires a large CASSCF calculation model, which is currently not possible with the existing computational possibilities. Unfortunately, the solution of the subsequent behaviour of the histidine molecule and the dimethylbenzimidazole ligand problem is very difficult, even if such a calculation would be theoretically possible, due to the lack of experimental data about the structure and the position of all the various substrates that could influence the behaviour of the histidine molecule and the dimethylbenzimidazole ligand at this stage. Considering that there are many such processes, 12 known so far [33], and their behaviour can be different, the solution to the problem is even more difficult. The experimental data [6,7] show that in the case of glutamate mutase and methylmalonyl-CoA mutase biochemical processes, the more convenient energetically chemical state is achieved in which the dimethylbenzimidazole ligand, after breaking the Co-N chemical bond at distances greater than 4.00 Å, interacts with various substrates and definitively separates from the cobalt atom. Finally, not only do the active substrates of the studied processes with the histidine molecule lead to the Co-N bond cleavage, but any substrate that contains the $–COO^-$ group together with the histidine molecule can also lead to the same result.

Conclusions

The adenosylcobalamin cofactor in Glutamate Mutase and Methylmalonyl-CoA Mutase bioprocesses, which are active in the human body, lose their dimethylbenzimidazole axial ligand in the preliminary phase of the enzymatically active act. The rupture of the Co-N axial bond and the removal of the axial ligand dimethylbenzimidazole take place under the joint influence of the histidine molecule, on the one hand, and of the negative ion of each bioactive substrate, which is participating in the enzymatic process. The process of the Co-N axial bond breaking occurs under the influence of partial electron density transfer from the highest occupied molecular orbitals HOMO1, HOMO2, and HOMO3, which are composed only of the atomic orbitals of the negative ion atoms of the substrates to the lowest unoccupied molecular orbitals LUMO1, LUMO2, and LUMO3, which represent antibonding molecular π-orbitals, composed from the atomic orbitals of the corrin ring and dimethylbenzimidazole ligand.

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