Research Article
Volume 11:8, 2021

Medicinal Chemistry

ISSN: 2161-0444 **Open Access**

The Electronic Structure and Mechanism of the Adenosylcobalamin-Dependent Bio-Processes as Determined by the MCSCF Method

Tudor Spataru*

Department of Chemistry, Columbia University, New York, USA

Abstract

The CASSCF geometry optimization of the adenosylcobalamin cofactor dependent processes common models with 12 orbitals and 12 electrons in the active space has been performed. The MCSCF geometry optimization shows a strong HOMO-LUMO coupling during the CASSCF orbitals mixing process. The HOMO-LUMO coupling causes an electronic density transfer from the HOMO, which at the beginning of the optimization process is constituted from the substrate atoms orbitals, to the LUMO, which is constituted from the adenosylco(III)balamin structure atomic orbitals. The Co-C bond cleavage reaction is starting from the beginning of the geometry optimization process due to the intermolecular transferred electronic density from the substrates to the adenosylco(III)balamin cofactor compound. Then, the HOMO and LUMO of the calculated models are converting into a bonding and an antibonding pair of orbitals with a central atom plus σ-axial ligands orbitals contribution and with a corrin ring plus axial ligands orbitals contribution, respectively. The HOMO-LUMO mixing process in the CASSCF procedure causes the intermolecular charge transfer process that converts into intramolecular charge transfer process, which is increasing up to about $1e^-$ at the Co-C bond cleavage distance. The substrates of the adenosylcobalamin cofactor dependent bio-processes from one side and the 5'- deoxy-5'-adenosyl radical from another side are permanently growing their direct interactions along with the Co-C bond rupture process up to a strong direct interaction at the Co-C bond cleavage distance. Evidently, this is allowing for a hydrogen atom transfer between them. Altogether, the total energy barrier of the hydrogen transfer reaction from the substrate to the 5'- deoxy-5'-adenosyl radical reaction, the CASSCF HOMO and LUMO surface orbitals of the substrate and 5'-adenosyl radical interaction common model before and after the hydrogen transfer and a strong Pseudo-Jahn-Teller effect for only direct reaction demonstrate that the hydrogen transfer is an irreversible tunneling process, which certainly leads to the final products. All these results are pointing out to the Co-C bond cleavage and hydrogen transfer from substrate to 5'- deoxy-5'-adenosyl ligand concerted reactions in full agreement with the experimental data.

Keywords

Amidalina • Medicinal plants • Anti malaria glutamate mutase • Methyleneglutarate mutase • Methylmalonyl adenosylcobalamin mutase • Ribonucleoside triphosphate reductase • CASSCF • Vitamin B12 • Electron transfer

Introduction

Adenosylcobalamin is a biologically active form of the vitamin B12. It catalyzes three classes of bioprocesses: skeleton mutases (Figure 1), amino mutases (Figure 2) and eliminases (Figure 3) [1-3]. The carbon skeleton mutase processes (Figure 1) catalyze the substrates modifications into their structural isomers. The amino mutase bioprocesses (Figure 2) catalyze the substrate 1-2 amino group transfer, while the eliminase processes catalyze the unusually isomer modifications and the elimination of small molecules from two adjacent $-NH_2$ and –OH, or –OH and -OH functional groups. The Ribonucleotide reductase process is not shown in these figures and will be treated additionally [4-8].

The adenosylcobalamin cofactor structure (Figure 4a) includes corrin ring as the equatorial ligand and two dimethylbenzimidazole and 5'- deoxy-5'-adenosyl as axial ligands, all coordinated to the Co^{+3} central ion. The charge of the central ion is balanced by -2 charge of the coordinated corrin ring and by -1 charge of the PO^{4-} group, which is present in the side chain of the adenosylcobalamin structure. The dimethylbenzimidazole ligand in adenosylcobalamin cofactor-dependent processes is replaced by the histidine ligand, as confirmed by kinetic and X-ray studies (Figure 4b) [9-13].

The starting reaction of these bio-processes has been considered to be the Co-C bond cleavage in the adenosylco(III)balamin cofactor to give rise to a 5'-deoxy-5'-adenosyl radical and to a co(II)balamin cofactor species. The involvement of the substrates into the Co-C bond cleavage process in adenosylcobalamin-dependent bio-processes has been proven by lots of experimental data, including by X-ray diffraction results [14-19]. On the other hand, the hydrogen transfer from the substrate to 5'-deoxy-5'-adenosyl radical has been proven to take place along with the Co-C bond cleavage in the adenosylcobalamin cofactor dependent bio-processes [20-25]. Many other effects like axial ligand trans-influence, steric effects, structural strain, protein influence and other effects have been considered to be connected to the Co-C cleavage process in adenosylcobalamine-dependent bio-processes although these last factors' participation into the Co-C bond cleavage has not been generally confirmed by subsequent experimental and theoretical studies [26-29].

The relationship between the Co-C bond cleavage and the transfer of a hydrogen atom from the substrate to the 5'-deoxy-5'-adenosyl radical processes has been the subject of theoretical studies that use QM/MM and DFT methodologies [30-34]. Generally, the QM/MM calculations show a lower total energy barrier for the Co-C bond cleavage reaction when compared to the total energy barrier for the Co-C bond cleavage process, which is calculated using DFT methods [35-38]. The causes of this difference in determining the energy barrier of total energy could be both objective and subjective factors that influence, jointly and separately, the total energy calculation results. One of the objective factors could be that the QM/MM method of calculation allows taking into account an important part of the substrate present in the MM structure [39-43]. Among the subjective factors, we can name the additional approximations included in the QM/MM calculations, which are not included in the DFT ones, such as the neglecting of a series of non-diagonal matrix elements at the boundary between the QM and MM section when heavier atoms are replaced by

***Address for Correspondence:** Dr. Tudor Spataru, Department of Chemistry, Columbia University, New York, New York 10027, USA; E-mail:ts2407@columbia.edu*

Received July 07, 2021; **Accepted** July 21, 2021; **Published** July 28, 2021

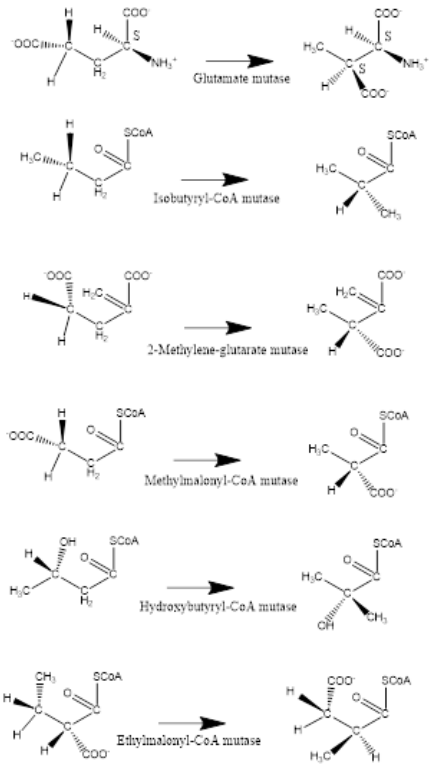

Figure 1. Bio-chemical reactions catalyzed by skeleton mutase bio-processes.

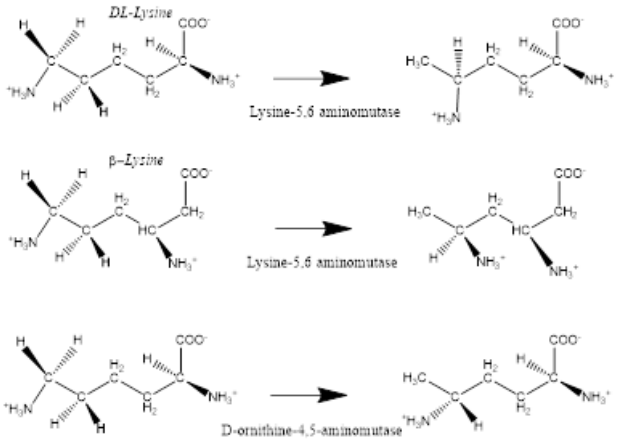

Figure 2. Bio-reactions catalyzed by aminomutase bio-processes.

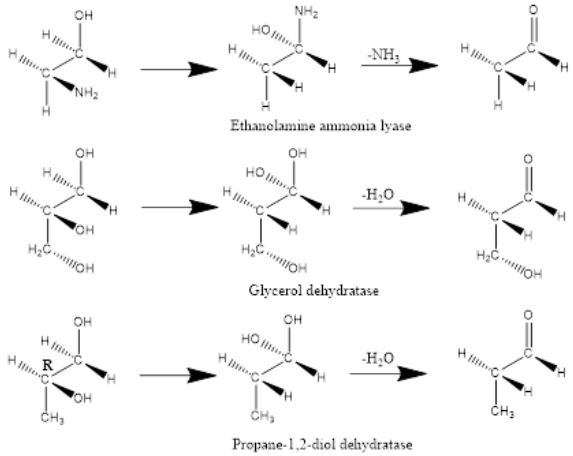

Figure 3. Bio-reactions catalyzed by eliminase bio-processes.

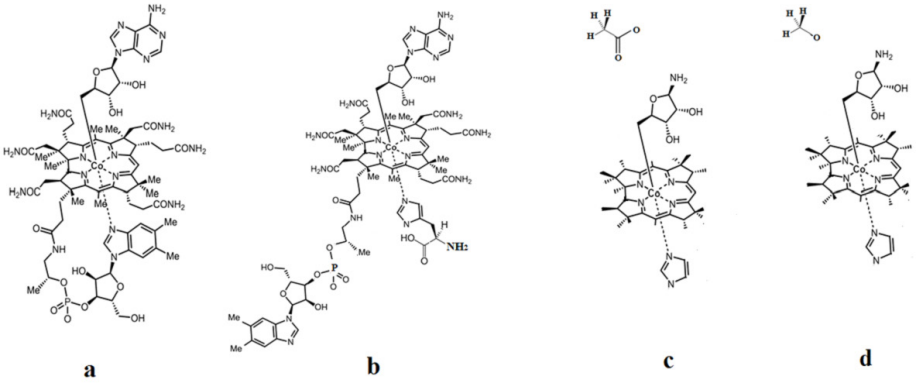

Figure 4. Structures of the adenosylcobalamin (a), the adenosylcobalamin with dimethylbenzimidazol ligand substituted by histidine (b) and the CASSCF electronic structure calculation models (c and d).

hydrogen atoms, the neglecting of the actual electronic structure of the atoms and their arbitrary considered electronic densities in the MM section of the QM/MM method, or the disproportions of the energies calculated by the MM method compared to the energies calculated by the QM method which, as a rule, is the major factor in the total energy barrier decrease in the QM/MM calculations due to the overcompensation of high QM section total energy barrier value by the low MM section total energy value [44-49].

In order to reduce the energy barrier of the DFT Co-C bond calculated breaking reaction, a possible reduction of the adenosylcob (III) alamin cofactor was assumed before the bio-processes have started [50-53]. However, in no theoretical calculation has the total energy barrier of the Co-C bond cleavage reaction in the adenosylco(III) balamin cofactor been fully eliminated in contradiction with the experimental data, which shows a close to the unity rate constant [5] (no energy barrier) even in the *in vitro* experiments (note that the solvent influences dramatically the rate constant of the Co-C bond cleavage process in a similar vitamin B12 cofactor compound [53,54]). Additionally, theoretical investigations did not find if the Co-C bond cleavage and the hydrogen transfer from substrate to 5'-adenosyl radical are concerted or stepwise reactions [44-47]. Therefore, new theoretical studies are necessary for the determination of the adenosylcobalamin cofactor dependent bio-processes mechanism.

Methodology

In our previous paper, we found the Methionine Synthase [55] process mechanism, which is apparently similar in the first step to the first step of the adenosylcobalamin cofactor dependent processes. We have shown that the dimethylbenzcobalamin ligand is substituted by histidine under the influence of substrate particles before the Methionine Synthase process starts. The resulting His-MeCbl(II) particles are participating into without-energy barrier Methionine Synthase process turnovers.

The adenosylcobalamin cofactor dependent processes have been studied extensively. Nevertheless, their detailed mechanism remains unknown in spite of the relevant number of researchers involved into their study. This paper is dedicated to the study of the adenosylcobalamin cofactor dependent bio-processes. More precisely, we are investigating the role of the substrates and the role of the hydrogen transfer from the substrates to the 5'deoxy-5'-adenosyl played in the Co-C bond cleavage process [56-58].

It has been proven that the coupling between occupied and unoccupied molecular orbital in cobalamin cofactors is of crucial importance in the mechanism of vitamin B12 dependent bio-processes [55, 57,58]. Bersuker has shown that the DFT (and QM/MM with DFT-QM section) method cannot be used to calculate the electronic structure of compounds, in which the mixing orbitals and the pseudo-Jahn-Teller effect are active [59-61]. Such systems go beyond the limits of DFT based methods. Therefore, throughout this study, we will use the MCSCF method.

Computational details

The CASSCF geometry optimization employs two models (Figures 4c and 4d) for the adenosylcobalamin dependent bio-processes. In our calculations, we used a 6-31G** basis set for the cobalt and oxygen atoms and a 6-31G basis set for the remainder of the atoms. We have shown that this is a satisfactory basis set for a similar cofactor dependent bio-process [55]. The NWChem computational software was used to optimize the geometry [62] of the CASSCF calculated models. We employed 12 electrons and 12 orbitals in the active zone of the CASSCF calculation procedure for the studied systems. A similar active MCSCF zone gave a correct determination of the Co-C bond cleavage process total energy in a similar bio-process [55]. On the other hand, our estimations have shown that an increase of the active zone does not lead to a change in the CASSCF(12,12) results. The embodied in the NwChem code, a quasi-newton optimization with line searches and approximate energy Hessian updates have been used for the geometry optimization procedure. The CASPT2, a more precise MCSCF procedure involving the dynamical correlation must be used, in principle, to complete the CASSCF calculations. Although the target of this study is the HOMO and LUMO electronic density and the position of the minimum of the studied reaction total energy, these characteristics are similar to the CASSCF and CASPT2 methods. Based on our knowledge, we have employed [55] one of the largest active zones for geometry optimization of such models. We believe that all the significant orbitals responsible for the studied reactions have been included in the calculations. The agreement between our calculations and the experimental data shows the correctness or our theoretical method. The Gaussian09 computational software was used for the geometry optimization of the starting geometry of the studied processes model employing a DFT approximation [63]. The B3LYP functional and 6-311++G** basis set has been used in the DFT calculations.

Results and Discussion

The subject of this study includes two main reactions of the studied bio-processes, Co-C bond cleavage and hydrogen transfer from the substrate to the 5'-deoxy-5'-adenosyl-axial-ligand of the adenosylco(III)balamin cofactor compound. We attempted to answer several questions: Are they concerted or step wise processes? What is the nature of the substrate participation along with the Co-C bond cleavage process? Is the hydrogen transfer from the substrate to 5'-deoxy-5'-adenosyl radical involved in the Co-C bond cleavage of the studied bio-processes?

In this study, we will arrange adenosylcobalamin dependent bio-processes in three new groups. In the first group, we will include the bioprocesses whose active substrates contain the $–COO^-$ group. In the second group, we will include the bioprocesses, the active substrates of which do not contain the $–COO^-$ group but contain the -OH group. In the last group, we will include the bioprocesses whose active substrates do not contain either the $–COO^-$ group or the -OH group.

The adenosylcobalamin dependent bioprocesses whose substrates contain –COO$^-$ group

This group includes Glutamate mutase, 2-Methylene-glutarate mutase, Methylmalonyl-CoA mutase, Ethylmalonyl-CoA mutase, two Lysine-5,6 aninomutases and D-ornithine-4,5 aminomutase bioprocesses (Figures 1 and 2). The substrate of the Ribonucleotide reductase (Ribonucleoside triphosphate reductase) process, which would be active along with the Co-C bond cleavage process, is still not determined experimentally. On the other hand, a very active catalytic substrate particle in this bioprocess is the cysteine anion, which contains the -COO$^-$ group and which, during the enzymatic process, is transferring hydrogen to the 5'-deoxy-5'-adenosyl radical (by homolytically breaking the -SH bond and formation of the thiyl radical) [42, 43]. Therefore, we also included in this group the Ribonucleoside Triphosphate Reductase as a process whose active substrate contains the –COO$^-$ group.

Apparently, one needs to employ two models in calculations involving the adenosylco(III)balamin cofactor and substrates joint structure to find out the mechanism of the Co-C bond cleavage as a main catalytic reaction of the studied adenosylco(III)balamin cofactor dependent processes. The first model would study the substrate influence on the Co-C cleavage reaction before the hydrogen transfer takes place from substrate to the 5'-deoxy-5'-adenosyl radical. The second model would include the transfer of the hydrogen to the 5'-deoxy-5'-adenosyl radical before or during the Co-C bond cleavage in order to study the influence of the hydrogen transfer from the substrate to the 5'-deoxy-5'-adenosyl radical on the Co-C bond rupture reaction. For this set of bioprocesses, we started with the system, which is modeling the substrate acting on the adenosylco(III)balamin cofactor compound before the Co-C bond is starting to be cleaved and before the hydrogen transfer from the substrate to the 5'-adenosyl radical takes place (Figure 4c). We used a CH3COO$^-$ group as the substrates model for these studied processes (Figure 4c). The side chains of the corrin ring were substituted with hydrogen atoms (Figure 4c). The dimethylbenzimidazole axial ligand was substituted with imidazole (Figure 4c), a model of the histidine ligand bounded to the cobalt central atom, as per the experimental data [1-4]. We used the main part of the 5'-deoxy-5'-adenosyl radical structure into these calculations (Figure 4c), replacing the rest of the 5'-deoxy-5'-adenosyl radical with hydrogen atoms. All these changes allowed us to take into account every responsible for the Co-C bond cleavage orbitals [64-68].

The CASSCF geometry optimization started at the axial Co-C and Co-N bond distances equal to 2.00 Å and to 2.12 Å, respectively. At this starting structure, the HOMO1, HOMO2 and HOMO3 of this adenosylcobalamin dependent processes group common model are the substrate atomic π-orbitals combinations, while LUMO1, LUMO2, LUMO3 and LUMO4 are adenosylco(III)balamin cofactor π-orbitals and σ-orbitals combinations (Figure 5). The energy distance between HOMO and LUMO at the Co-C bond distance of 2.00 Å is equal to about 8.17 eV, while the population of the HOMO and LUMO is equal to about 1.79e$^-$ and 0.21e$^-$, respectively. The results show that the population of LUMO increases by an amount equal to the electronic density transfer from the HOMO due to the HOMO-LUMO coupling in the CASSCF procedure orbitals mixing. The CASSCF geometry optimization process lead to the Co-C distance permanent increasing from 2.00 Å through 3.17 Å (and up to a larger Co-C distance), gradually releasing the 5'-deoxy-5'-adenosyl radical and co(II)abalamin cofactor particle. The intermolecular electronic density transfer from the HOMO to the LUMO is increasing constantly from 0.21e- at the 2.00 Å of the Co-C bond distance to 0.56e- at the 2.50 Å of the Co-C bond distance (Figures 5 and 6). Then, the highest occupied molecular orbital starts its converting from the π-substrate atomic orbitals combinations to the bonding orbital with a major contribution of the central and axial atoms σ-orbitals while LUMO is starting its converting from π-corrin ring orbitals to an antibonding orbital with a π-corrin ring, central atom and an axial ligands atomic σ-orbitals combination. Their full conversion is finished at about 2.65 Å of the Co-C bond distance (Figure 6). The intermolecular charge transfer from the substrate to the adenosylco(III) balamin cofactor compound is diminishing and the charge transfer from HOMO to LUMO becomes intramolecular (Figure 6). Concomitantly, the HOMO-LUMO coupling degree is increasing, which stimulates an internal charge transfer increase and the cleavage of the Co-C bond.

From the Co-C bond distance equal to about 2.65 Å up to the Co-C bond distance equal to about 3.17 Å, the sum of the intramolecular electronic density transfer from the four highest occupied molecular orbitals to the four lowest unoccupied molecular orbitals increases up to an entire

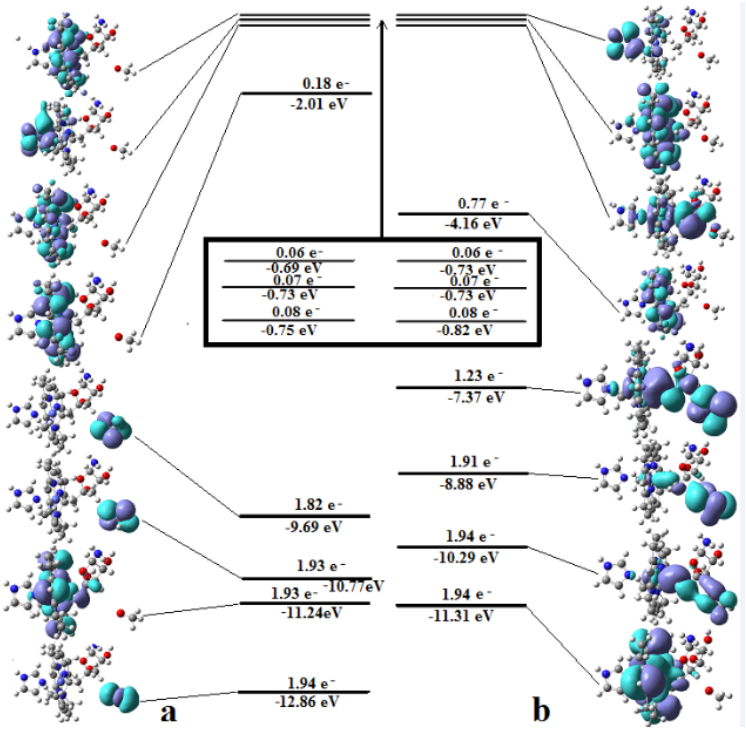

Figure 5. CASSCF HOMO-LUMO surfaces, the behavior of the HOMO-LUMO levels energy and their population in the adenosylco(III) balamin cofactor dependent bio-processes used model: a) The Co-C bond distance is equal to 2.00 Å; b) The Co-C bond distance is equal to 3.17 Å.The data inside the rectangle refers to the LUMO2, LUMO3 and LUMO4 characteristics.

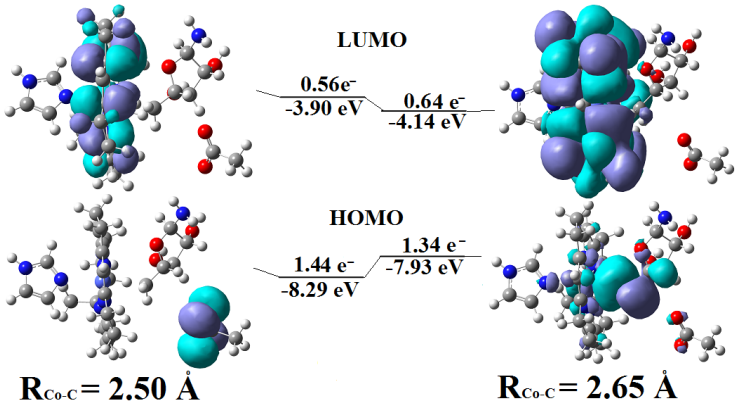

Figure 6. CASSCF HOMO-LUMO surfaces, the behavior of the HOMO-LUMO levels energy and their population in the adenosylco(III) balamin cofactor dependent bio-processes used model: The Co-C bond distances are equal to 2.50 Å (left side) and 2.65 Å (right side).

electron (Figure 5). At the same time, the HOMO-LUMO distance decreases from about 8.17 eV at the beginning of the CASSCF geometry optimization process to about 2.89 eV at the Co-C bond distance of about 3.17 Å. This suggests that at the beginning of the reaction, the substrate transfers an amount of the electronic density to the adenosylco(III)balamin cofactor particle from about 0.21e- at 2.00 Å of the Co-C bond distance to about 0.56e- at about 2.50 Å of the Co-C bond distance. This electronic density transfer triggers the Co-C bond cleavage process.

Then, starting at about 2.50 Å of the Co-C bond distance, the intermolecular electronic density transfer from the bonding HOMO to anti-bonding LUMO decreases, while the intramolecular electronic density transfer from the bonding HOMO to anti-bonding LUMO increases due to their larger mixing degree leading to a near full Co-C bond cleavage at the Co-C bond distance of about 3.17 Å.

In conclusion, the interaction of the HOMO-LUMO orbitals and the charge transfer from HOMO to LUMO leads to a near full Co-C bond distance cleavage. The further geometry optimization and implicitly the Co-C bond cleavage processes are connected to the strong substrate and the 5'-deoxy-5'-adenosyl axial ligand interaction.

It is interesting to observe the modification of the distance between the substrate model and the 5'-deoxy-5'-adenosyl radical along with the modification of the Co-C bond distance. At the beginning of the CASSCF geometry optimization (at the 2.00 Å of the Co-C bond distance), the substrate model is situated at the distance of about 5.00 Å from the closest 5'-deoxy-5'-adenosyl ligand atoms. As the geometry optimization procedure is running out, the Co-C bond distance increases, while the distance between the substrate model and the 5'-deoxy-5'-adenosyl radical decreases concomitantly because of the Co-C bond distance increase and of the 5'-deoxy-5'-adenosyl radical position orientation toward the substrate model. At about 3.17 Å of the Co-C bond distance, the substrate model is situated at about 2.11 Å from the 5'deoxy-5'-adenosyl radical. At the same Co-C bond distance (3.17 Å), the 5'-deoxy-5'-adenosyl radical interaction with the substrate becomes strong, all four HOMO of the calculated common model became common 5'-deoxy-5'-adenosyl radical and substrate intermolecular molecular orbitals (Figure 5). This shows the strong connection between the gradual breaking of the Co-C bond and the gradual increasing interaction of the substrates with the gradually formed 5'-deoxy-5'-adenosyl radical. Obviously, the strong interaction between the substrate and the 5'-deoxy-5'-adenosyl radical at the Co-C bond distance of 3.17 Å is naturally creating favorable conditions for the hydrogen transfer from the substrate to the 5'-deoxy-5'-adenosyl radical. Once the hydrogen transfer from the substrate to the 5'-deoxy-5'-adenosyl radical takes place, the full Co-C bond cleavage is finished along with the hydrogen transfer reaction. In conclusion, the transfer of hydrogen from substrates to the 5'-deoxy-5'-adenosyl radical is so closely related to the rupture process of the Co-C bond that cannot occur without it. Accordingly, these two reactions cannot be, in principle, separated by the experiment, showing why the free 5'-deoxy-5'-adenosyl radical was not found in all experimental data up to now. In other words, these two processes can be considered as mutually dependent concerted reactions. The common mechanism of these two reactions is drawn in Figure 7.

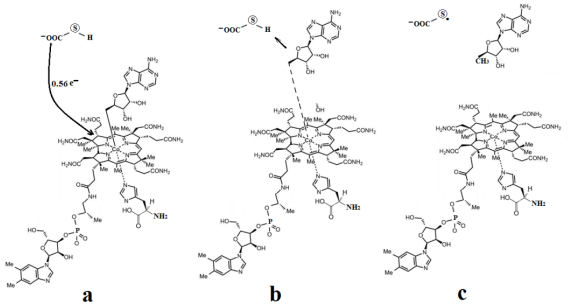

Figure 7. The mechanism of the Co-C bond cleavage and of the hydrogen transfer from substrate (H(S)COO· model) to the 5'-deoxy-5'-adenosyl radical in the adenosylcobalamin dependent processes: a) The initial electronic density transfer from substrate to adenosylco(III)balamin cofactor; b) The Co-C bond cleavage in the adenosylcobalamin cofactor under influence of the substrate; c) The hydrogen tunneling between substrate and the 5'-deoxy-5'-adenosyl radical.

The adenosylcobalamin dependent bioprocesses whose substrates contain –COH group

This group includes Hydroxybutyryl-CoA Mutase, Ethanolamine ammonia mutase, Glycerol dehydratase and Propane-1,2-diol dehydratase bioprocesses (Figures 1 and 3). Although the substrates of these bioprocesses contain the -OH group instead of the –COO- group, the experimental data shows that their mechanism is similar. It is commonly known that the largest pKa value of alcohols is equal to about 18. The primary alcohols pKa values are equal to about 10. It is also well known that in the presence of the R–NH^{3+} groups, which pKa is equal to about 35, the R –OH group loses its proton and becomes a R-O- group. It is also well known that the cavity of the adenosylcobalamin cofactor structure includes a large number of R-NH^{3+} groups. Following this, we can consider that the R-OH group can act as a R-O- group in the mechanism of the adenosylcobalamin dependent bioprocesses. As in the case of the first group of bioprocesses, we started the CASSCF geometry optimization with the system, which is modeling the substrate acting on the adenosylco(III) balamin cofactor compound before the Co-C bond starts to be cleaved and before the hydrogen transfer from the substrate to the 5'-deoxy-5'-adenosyl radical takes place (Figure 4d). We used a CH_3O^- group as the substrates model of these processes (Figure 4d). The rest of this model simplification is similar to the previous model simplifications.

The CASSCF geometry optimization started at the axial Co-C and Co-N bond distances equal to 2.00 Å and to 2.12 Å, respectively. At this starting structure, the HOMO1, HOMO2 and HOMO4 of this adenosylcobalamin dependent processes group common model are the substrate atomic π-orbitals combinations, while LUMO1, LUMO2, LUMO3 and LUMO4 of this adenosylcobalamin dependent group processes common model are the adenosylco(III)balamin cofactor π- and σ-orbitals combinations (Figure 8). The distance between HOMO and LUMO energies at the Co-C bond distance of 2.00 Å is equal to about 7.68 eV, while the population of the HOMO and LUMO is equal to about 1.82e^- and 0.18e^-, respectively. It follows that the population of LUMO increases with the amount equal to the electronic density transfer from the HOMO due to the HOMO-LUMO coupling in the CASSCF procedure orbitals mixing. The CASSCF geometry optimization leads to the Co-C distance permanent increase from 2.00 Å to 3.05 Å (and up to a larger Co-C distance), while gradually releasing the 5'-deoxy-5'-adenosyl radical and co(II)abalamin cofactor particle. The intermolecular electronic density transfer from HOMO to the LUMO increases constantly from 0.18e- at the 2.00 Å of the Co-C bond distance to the amount equal to about 0.77e- at the 3.05 Å of the Co-C bond distance (Figure 8). In this case, unlike the behavior of the first set of bioprocesses model, the electron density transfer from the substrate to the adenosylcobalamin cofactor does not change from intermolecular to intramolecular, but remains intermolecular throughout the whole CASSCF geometry optimization process. Only at the Co-C bond distance of about 3.00 Å are the HOMO and LUMO orbitals are starting their converting in the same way as in the case of the model of the first set of bioprocesses. Also, the intermolecular electron density transfer between substrate and the cofactor adenosylcobalamin converts from intermolecular to intramolecular transfer (Figure 8).

The HOMO-LUMO coupling degree increases permanently during the geometry optimization process. This stimulates the intermolecular charge transfer increase and the cleavage of the Co-C bond. At the Co-C bond distance equal to about 3.05 Å, the sum of the electronic density transfer from the four highest occupied molecular orbitals to the four lowest unoccupied molecular orbitals increases up to about one electron (Figure 8). At the same time, the HOMO-LUMO distance decreases from about 7.68 eV at the beginning of the CASSCF geometry optimization process to about 3.21 eV at the Co-C bond distance equal to about 3.05 Å. It follows that the HOMOs with substrate orbitals participation transfer about one electron to the LUMOs with adenosylcobalamin cofactor orbitals participation during the geometry optimization process. This electronic density transfer leads to the near full Co-C bond cleavage. The further geometry optimization and implicitly, the Co-C bond cleavage processes are connected to the strong substrate and the 5'-deoxy-5'-adenosyl axial ligand interaction.

As in the previous model, at the beginning of the geometry optimization, the substrate model is situated at the distance of about 5.00 Å from the closest of the 5'-deoxy-5'-adenosyl ligand atoms. As the geometry optimization procedure is running out, the Co-C bond distance increases, while the distance between the substrate model and the 5'-deoxy-5'-adenosyl radical decreases because of the Co-C bond distance increase and because of the 5'-deoxy-5'-adenosyl radical position orientation towards the substrate model. At about 3.05 Å of the Co-C bond distance, the substrate model is situated at about 1.86 Å from the 5'-deoxy-5'-adenosyl radical. At the same Co-C bond distance, the 5'-deoxy-5'-adenosyl radical interaction with the substrate model becomes strong. Three HOMO of the full calculated common model become common intermolecular molecular orbitals (Figure 8).

Again, this shows the strong connection between the gradual Co-C bond cleavage process and the increasing interaction between the substrates and the gradually formed 5'-deoxy-5'-adenosyl radical. Obviously, the strong interaction between the substrate and the 5'-deoxy-5'-adenosyl radical at the Co-C bond distance of 3.05 Å is creating the favorable conditions for the hydrogen transfer from the substrate to the 5'-deoxy-5'-adenosyl radical. Once the hydrogen transfer from the substrate to the 5'-deoxy-5'-adenosyl radical takes place, the full Co-C bond cleavage is finished along with the hydrogen transfer reaction. In conclusion, the transfer of hydrogen from substrates to the 5'-deoxy-5'-adenosyl radical is closely related to the rupture process of the Co-C bond that cannot occur without it. As was the case with the first set of bioprocesses, for this set of bioprocesses the Co-C bond cleavage and the hydrogen transfer from the substrates to the 5'-deoxy-5'-adenosyl radical reactions cannot be separated by the experiment showing why the free 5'-deoxy-5'-adenosyl radical was not found in all experimental data up to now. In other words, these two processes can be considered as mutually dependent concerted reactions. The common mechanism of these two reactions is drawn in Figure 9.

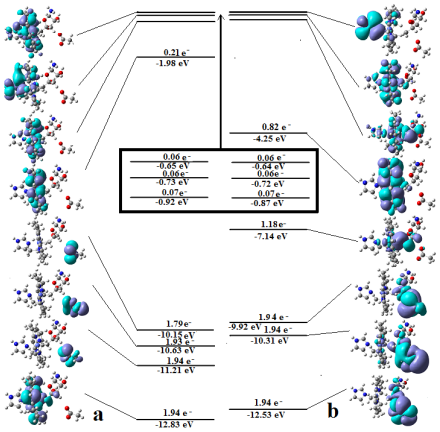

Figure 8. CASSCF HOMO-LUMO surfaces, the behavior of the HOMO-LUMO levels energy and their population in the adenosylco(III)balamin cofactor dependent bio-processes used model: a) The Co-C bond distance is equal to 2.00 Å; b) The Co-C bond distance is equal to 3.05 Å. The data inside the rectangle refers to the LUMO2, LUMO3 and LUMO4 characteristics.

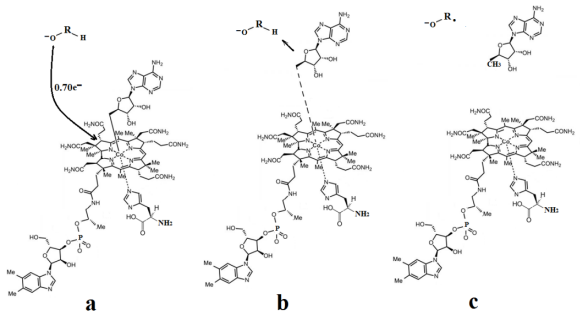

Figure 9. The mechanism of the Co-C bond cleavage and of the hydrogen transfer from substrate (RO- model) to the 5'-deoxy-5'-adenosyl -radical in the adenosylcobalamin dependent processes: a) The initial electronic density transfer from substrate to the adenosylco(III)balamin cofactor; b) The Co-C bond cleavage in the adenosylcobalamin cofactor under the influence of the substrate; c) The hydrogen tunneling between substrate and the 5'-deoxy-5'-adenosyl radical.

The adenosylcobalamin dependent bioprocesses whose substrates contain neither the –COO- group nor the -OH group

Only Isobutyryl-CoA mutase bioprocess belong to this group (Figure 1). We did not treat beyond the Isobutyryl-CoA Mutase process for the simple reason that, apparently, the substrate of this bio-process does not have the active R-COO- or R-O- groups in its structure.

In fact, the radical of amino acid valine, which has the COO- in its structure [67], is the substrate that participates into the catalytic process with the Co-C bond cleavage of the adenosylcobalamin cofactor, as shown in [67]. Then, the valine radical activates the main substrate of the Isobutyryl-CoA mutase bio-process [64] (Figure 10). In conclusion, all adenosylcobalamin dependent bioprocesses, including the Isobutyryl-CoA Mutase process, are following a similar mechanism (Figures 7 and 9).

The hydrogen transfer from the adenosylcobalamin co-factor dependent bioprocesses substrates to the 5'-de-oxy-5'-adenosyl radical

Another particularity of the quantum tunneling effect is the initial compounds wave functions penetrating the energy barrier from one end to the other. Our calculations show that their nature and particularities are extremely similar, so we are presenting here the detailed analysis of one of them (Figure 11). Figure 11 represents the surfaces of the HOMO and LUMO orbitals for the 1,2-propanediol (the substrate of the Propane-1,2 dehydratase bioprocess) and the 5'-deoxy-5'-adenosyl radical common model at the beginning of the hydrogen transfer process. The nature of these molecular orbitals is somewhat similar. Due to the CASSCF mixing procedure, their electron population is also very similar, each of them being equal to about one electron. A very important feature of these molecular orbitals is their location in the space between the reactants, which is exactly in the direction of hydrogen transfer from one compound to another, fully penetrating the entire total energy barrier of the hydrogen transfer reaction. Another important feature of these frontier orbitals is that they include mainly the orbitals of three atoms involved in hydrogen tunneling, of the carbon atoms of both systems participating in the reaction, from which the hydrogen transfer takes place and to which the hydrogen is transferred, even if the distance between them exceeds 4.10 A and especially of the hydrogen atom, which is transferred from one system to another. All this is satisfying the requirements imposed by the nature of hydrogen tunneling transfer.

It is interesting to observe nature of the frontier orbitals of the substrates of the studied here bioprocesses and 5'-deoxy-5'-adenosyl radical common model at the end of the hydrogen transfer process. Our calculations show that their nature and particularities are similar, so we are presenting here the detailed analysis of one of them (Figure 12). Figure 12 represents the surfaces of the HOMO and LUMO orbitals for the 2-methylene-glutarate (the substrate of the 2-methylene-glutarate-mutase bioprocess) and the 5'-deoxy-5'-adenosyl radical common model at the end of the hydrogen transfer process. The nature of these molecular orbitals is not similar. As a result, the CASSCF mixing procedure did not transfer much of the HOMO electron density to LUMO (only about 0.07 e-), so their electron density is quite different. A very important feature of these molecular orbitals is their composition from the internal reactant atoms orbitals. Also, they are not situated on the space between the reactants and is not penetrating the total energy barrier of the hydrogen transfer reaction. Another important feature of these frontier orbitals is that they do not include the orbitals of all three atoms involved in hydrogen tunneling, of the carbon atoms of both systems participating in the reaction, from which the hydrogen transfer takes place and to which the hydrogen is transferred and of the hydrogen atom, which is transferred from one system to another. All this is not satisfying the requirements imposed by the nature of reverse hydrogen tunneling transfer.

It has been shown, that the hydrogen tunneling effect can be treated in the framework of the pseudo Jahn-Teller-Effect [59-61, 67, 68], which destabilizes the system under study and can lead to the breaking of some chemical bonds and the formation of others. As shown above, the pseudo Jahn-Teller-Effect is very strong for the direct reaction of hydrogen transfer from the substrates of adenosylcobalamin cofactor dependent biochemical processes to the 5'-deoxy-5'-adenosyl radical. At the same time the pseudo Jahn-Teller-Effect is very weak or even absent for the reverse reactions. In conclusion, the presence of a strong pseudo Jahn-Teller-Effect is supporting the hydrogen tunneling in the direct reaction and the lack of a Jahn-Teller-Effect pseudo does not support the reverse tunneling reaction.

As mentioned above, all the characteristics of hydrogen transfer reactions from the substrates to the 5'deoxy-5'-adenosyl radical (the height of the energy barrier, the nature of frontier orbitals and the effective pseudo Jahn-Teller-Effect for only direct reaction) are similar for all adenosylcobalamin dependent studied processes and are supported the its tunneling nature. In conclusion, our data proves that the hydrogen transfer from the substrates of the adenosylcobalamin dependent bioprocesses to the 5'-deoxy-5'-adenosyl radical is an irreversible tunneling reaction, ensuring the completeness of the process.

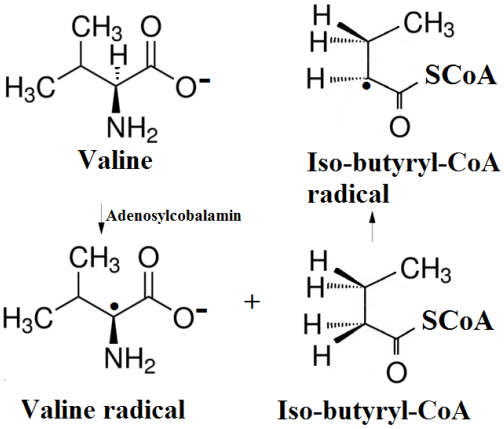

Figure 10. The Isobutyryl-CoA Mutase process substrate activation by valine radical after its catalytic activation by adenosylcobalamin cofactor.

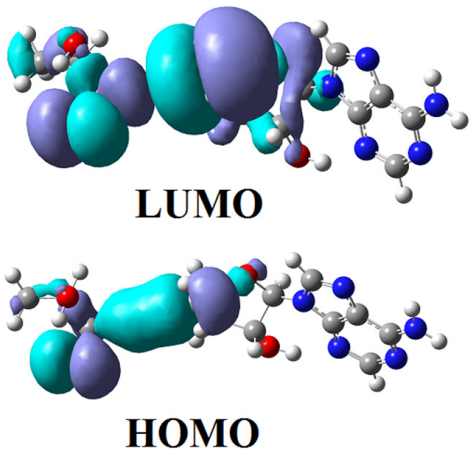

Figure 11. CASSCF HOMO-LUMO surfaces of the 1, 2-propanediol and the 5'deoxy-5'-adenosyl radical common model at the beginning of the hydrogen transfer process

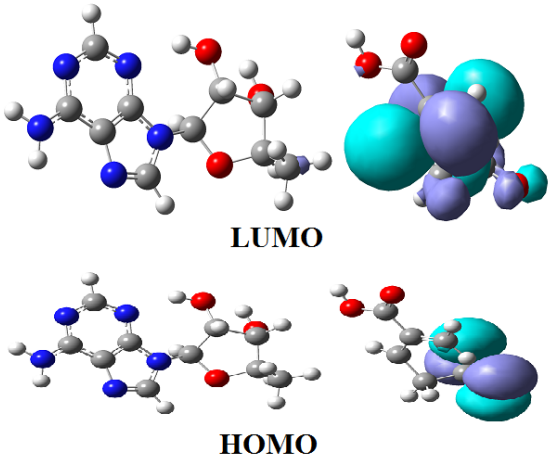

Figure 12. CASSCF HOMO-LUMO surfaces of the 2-methylene-glutarate and the 5'deoxy-5'-adenosyl radical common model at the beginning of the hydrogen transfer process.`

Conclusion

The adenosylcobalamin cofactor dependent bioprocesses models CASSCF geometry optimization lead to the without energy barrier Co-C bond cleavage. The Co-C cleaved bond corresponds to the minimal total energy of the used models. The triggering factor of the Co-C bond cleavage process is the intermolecular electronic density transfer from the substrates of the adenosylcobalamin dependent processes to the adenosylco(III) balamin cofactor itself starting from the beginning and increasing during the CASSCF optimization process. The strong HOMO-LUMO coupling and the entire electron intramolecular charge transfer from bonding HOMOs to antibonding LUMOs in the adenosylcobalamin dependent bioprocesses models lead to the full Co-C bond cleavage and to the substrates of the studied processes with the 5'-deoxy-5'-adenosyl radical strong interaction. The CASSCF calculations of the adenosylcobalamin cofactor dependent bioprocesses substrates and the 5'-deoxy-5'-adenosyl radical common model by using the 6-31G** basis set for all atoms and 10 electrons and 10 orbitals active zone have shown that the hydrogen transfer from the adenosylcobalamin cofactor dependent bioprocesses substrates to the 5'-deoxy-5'-adenosyl radical is an irreversible tunneling process. The Co-C bond cleavage in studied adenosylcobalamin cofactor dependent bioprocesses and the hydrogen transfer from their substrates to the 5'-deoxy-5'-adenosyl radical are mutually dependent concerted reactions in full agreement with the experimental data.

Acknowledgment

This research was supported, in part, under National Science Foundation Grants CNS-0958379 and CNS0855217 and the City University of New York High Performance Computing Center at the College of Staten Island and by the National Science Foundation through TeraGrid resources provided by the TeraGrid Science Gateways program under grants CHE090082 and CHE140071.

References

1. Marsh E, Neig G. "Review Article Coenzyme-B12-Dependent Glutamate Mutase." *Bioorg Chem* 28 (2000): 176-189.
2. Kräutler, Bernard, Arigoni D, Golding B.T. Eds. Vitamin B12 and the B12 Proteins. Innsbruck: Wiley-VCH: Weinheim, Chapter 12, Austria, (1998).
3. Babior, Bernard M, Thomas J. Carty and Robert H. Abeles. "The Mechanism of Action of Ethanolamine Ammonia-Lyase, a B12-Dependent Enzyme: The Reversible Formation of 5'-Deoxyadenosine from Adenosylcobalamin during the Catalytic Process." *J Biol Chem* 249 (1974): 1689-1695.
4. Toraya, Tetsuo. "Radical Catalysis of B12 Enzymes: Structure, Mechanism, Inactivation and Reactivation of Diol and Glycerol Dehydratases." *Cell Mol Life Sci* 57 (2000): 106-127.
5. Marsh, E. Neil G and David P. Ballou. "Coupling of Cobalt–Carbon Bond Homolysis and Hydrogen Atom Abstraction in Adenosylcobalamin-Dependent Glutamate Mutase." *Biochem* 37 (1998): 11864-11872.
6. Chowdhury, Shantanu and Ruma Banerjee. "Thermodynamic and Kinetic Characterization of Co– C Bond Homolysis Catalyzed by Coenzyme B12-Dependent Methylmalonyl-CoA Mutase." *Biochem* 39 (2000): 7998-8006.
7. Gruber, Karl, Riikka Reitzer and Christoph Kratky. "Radical Shuttling in a Protein: Ribose Pseudorotation Controls Alkyl-Radical Transfer in the Coenzyme B12 Dependent Enzyme Glutamate Mutase." *Angew Chem Int Ed* 40 (2001): 3377-3380.
8. Reitzer, Riikka, K. Gruber, G. Jogl and U. G. Wagner, et al. "Glutamate Mutase from Clostridium Cochlearium: the Structure of a Coenzyme B12-Dependent Enzyme Provides New Mechanistic Insights." *Structure* 7 (1999): 891-902.
9. Kräutler, Bernard, Arigoni D and Golding Bernard T. Eds. Vitamin B12 and the B12 Proteins. Innusbruck: Wiley-VCH: Weinheim, Austria, Chapter 16, (1998).
10. Buckel, Wolfgang, Golding and Bernard T. *Wiley Encyclopedia of Chemical Biology*. Marburg: Wiley & Sons, Germany, (2008).
11. Banerjee, Ruma and Stephen W. Ragsdale. "The Many Faces of Vitamin B12: Catalysis by Cobalamin-Dependent Dnzymes." *Annu Rev Biochem* 72 (2003): 209-247.
12. Yamanishi, Mamoru, Tetyana Labunska and Ruma Banerjee. "Mirror "Base-Off" Conformation of Coenzyme B12 in Human Adenosyltransferase and its Downstream Target, Methylmalonyl-CoA Mutase." *J Am Chem Soc* 127 (2005): 526-527.
13. Brooks, Amanda J, Monica Vlasie, Ruma Banerjee and Thomas C. Brunold. "Co– C Bond Activation in Methylmalonyl-CoA Mutase by Stabilization of the Post-Homolysis Product Co2+ Cobalamin." *J Am Chem Soc* 127 (2005): 16522-16528.
14. Buckel, Wolfgang, Christoph Kratky and Bernard T. Golding. "Stabilisation of Methylene Radicals by Cob (II) Alamin in Coenzyme B12 Dependent Mutases." *Chem Eur J* 12 (2005): 352-362.
15. Madhavapeddi, Prashanti, David P. Ballou and Marsh E, Neil G. "Pre-Steady-State Kinetic Studies on the Glu171Gln Active Site Mutant of Adenosylcobalamin-Dependent Glutamate Mutase." *Biochem* 41 (2002): 15803-15809.
16. Pallares, Ivan G, Theodore C. Moore, Jorge C. Escalante-Semerena and Thomas C. Brunold. "Spectroscopic Studies of the Salmonella Enterica Adenosyltransferase Enzyme Se CobA: Molecular-Level Insight into the Mechanism of Substrate Cob (II) Alamin Activation." *Biochem* 53 (2014): 7969-7982.
17. Brown, Kenneth L and Jing Li. "Activation Parameters for the Carbon–Cobalt Bond Homolysis of Coenzyme B12 Induced by the B12-Dependent Ribonucleotide Reductase from Lactobacillus leichmannii." *J Am Chem Soc* 120 (1998): 9466-9474.
18. Moore, Theodore C, Sean A. Newmister, Ivan Rayment and Jorge C. Escalante-Semerena. "Structural Insights into the Mechanism of Four-Coordinate Cob (II) Alamin Formation in the Active Site of the Salmonella Enterica ATP: Co (I) Rrinoid Adenosyltransferase Enzyme: Critical Role of Residues Phe91 and Trp93." *Biochem* 51 (2012): 9647-9657.
19. Marsh E, Neil G. "Adenosylcobalamin Enzymes: Theory and Experiment Begin to Converge." *Biochim Biophys Acta* 1824 (2012): 1154-1164.
20. Padmakumar, Rugmini, Raghavakaimal Padmakumar and Ruma Banerjee. "Evidence that Cobalt–Carbon Bond Homolysis is Coupled to Hydrogen Atom Abstraction from Substrate in Methylmalonyl-CoA Mutase." *Biochem* 36 (1997): 3713-3718.
21. Marsh E, Neil G and David P. Ballou. "Coupling of Cobalt–Carbon Bond Homolysis and Hydrogen Atom Abstraction in Adenosylcobalamin-Dependent Glutamate Mutase." *Biochem* 37 (1998): 11864-11872.
22. Babior, Bernard M, Thomas H. Moss, Orme-Johnson William and Beinert Helmut, "The Mechanism of Action of Ethanolamine Ammonia-Lyase, a B-12-Dependent Enzyme. The Participation of Paramagnetic Species in the Catalytic Deamination of 2-Aminopropanol." *J Biol Chem* 249 (1974): 4537-4544.
23. Licht, Stuart S, Squire Booker and JoAnne Stubbe. "Studies on the Catalysis of Carbon– Cobalt Bond Homolysis by Ribonucleoside Triphosphate Reductase: Evidence for Concerted Carbon–Cobalt Bond Homolysis and Thiyl Radical Formation." *Biochem* 38 (1999): 1221-1233.
24. Meier, Thomas W, Nicolas H. Thomä and Peter F. Leadlay. "Tritium Isotope Effects in Adenosylcobalamin-Dependent Methylmalonyl-CoA Mutase." *Biochemistry* 35 (1996): 11791-11796.
25. Chih, Hung-Wei and Marsh E, Neil G. "Pre-Steady-State Kinetic Investigation of Intermediates in the Reaction Catalyzed by Adenosylcobalamin-Dependent Glutamate Mutase." *Biochem* 38 (1999): 13684-13691.
26. Bandarian, Vahe and George H. Reed. "Isotope Effects in the Transient Phases of the Reaction Catalyzed by Ethanolamine Ammonia-Lyase: Determination of the Number of Exchangeable Hydrogens in the Enzyme–Cofactor Complex." *Biochem* 39 (2000): 12069-12075.
27. Kräutler, Bernhard, Arigoni D and Golding Bernard T. Eds. Vitamin B12 and the B12 Proteins. Innsbruck: Wiley-VCH: Weinheim, Austria, (1998).

28. Banerjee, Ruma. "The Yin-Yang of Cobalamin Biochemistry." *Chemistry & biology* 4 (1997): 175-186.

29. Miri, Yoon, Hangtian Song, Kristina Hakansson and E. Neil G. Marsh. "Hydrogen Tunneling in Adenosylcobalamin-Dependent Glutamate Mutase: Evidence from Intrinsic Kinetic Isotope Effects Measured by Intramolecular Competition." *Biochem* 49 (2010): 3168-3173.

30. Hayward, G. C, H. A. O. Hill, J. M. Pratt and N. J. Vanston, et al. "The Chemistry of Vitamin B 12. Part IV. The Thermodynamic Trans-Effect." *J Chem Soc* (1965): 6485-6493.

31. Grate, John H and G. N. Schrauzer. "Chemistry of Cobalamins and Related compounds. 48. Sterically Induced, Spontaneous Dealkylation of Secondary Alkylcobalamins due to Axial Base Coordination and Conformational Changes of the Corrin Ligand." *J Am Chem Soc* 101 (1979): 4601-4611.

32. Halpern, Jack. "Mechanisms of Coenzyme B12-Dependent Rearrangements." *Science* 227 (1985): 869-875.

33. Marsh E, Neil G and Catherine L. Drennan. "Adenosylcobalamin-Dependent Isomerases: New Insights into Structure and Mechanism." *Curr Opin Chem Biol* 5 (2001): 499-505.

34. Brown, Kenneth L and Xiang Zou. "Thermolysis of Coenzymes B12 at Physiological Temperatures: Activation Parameters for Cobalt–Carbon Bond Homolysis and a Quantitative Analysis of the Perturbation of the Homolysis Equilibrium by the Ribonucleoside Triphosphate Reductase from Lactobacillus leichmannii." *J Inorg Biochem* 77 (1999): 185-195.

35. Dong, Shoulian, Raghavakaimal Padmakumar, Ruma Banerjee and Thomas G. Spiro. "Co– C Bond Activation in B12-Dependent Enzymes: Cryogenic Resonance Raman Studies of Methylmalonyl-Coenzyme A Mutase." *J Am Chem Soc* 121 (1999): 7063-7070.

36. Bresciani-Pahor, Nevenka, Margherita Forcolin, Luigi G. Marzilli and Lucio Randaccio, et al. "Organocobalt B12 Models: Axial ligand Effects on the Structural and Coordination Chemistry of Cobaloximes." *Coord Chem Rev* 63 (1985): 1-125.

37. Mancia, Filippo and Philip R. Evans. "Conformational Changes on Substrate Binding to Methylmalonyl CoA Mutase and New Insights into the Free Radical Mechanism." *Structure* 6 (1998): 711-720.

38. Vlasie, Monica D and Ruma Banerjee. "Tyrosine 89 Accelerates Co– Carbon Bond Homolysis in Methylmalonyl-CoA Mutase." *J Am Chem Soc* 125 (2003): 5431-5435.

39. Thomä, Nicolas H, Thomas W. Meier, Philip R. Evans and Peter F. Leadlay. "Stabilization of Radical Intermediates by an Active-Site Tyrosine Residue in Methylmalonyl-CoA Mutase." *Biochem* 37 (1998): 14386-14393.

40. Toraya, Tetsuo and Atsuhiko Ishida. "Acceleration of Cleavage of the Carbon-Cobalt Bond of Sterically Hindered Alkylcobalamins by Binding to Apoprotein of Diol Dehydrase." *Biochem* 27 (1988): 7677-7681.

41. Zhu, Longgen and Nenad M. Kostic. "Molecular Orbital Study of Coenzyme B12. Activation of the Cobalt-Carbon Bond by Angular Distortions." *Inorg Chem* 26 (1987): 4194-4197.

42. Stuart S, Licht, Squire Booker and JoAnne Stubbe. "Studies on the Catalysis of Carbon– Cobalt Bond Homolysis by Ribonucleoside Triphosphate Reductase: Evidence for Concerted Carbon–Cobalt Bond Homolysis and Thiyl Radical Formation." *Biochem* 38 (1999): 1221-1233.

43. Stuart S, Licht, Christopher C. Lawrence and JoAnne Stubbe. "Thermodynamic and Kinetic Studies on Carbon– Cobalt Bond Homolysis by Ribonucleoside Triphosphate Reductase: The Importance of Entropy in Catalysis." *Biochem* 38 (1999): 1234-1242.

44. Jensen, Kasper P and Ulf Ryde. "How the Co– C Bond is Cleaved in Coenzyme B12 Enzymes: A Theoretical Study." *J Am Chem Soc* 127 (2005): 9117-9128.

45. Kwiecien, Renata A, Ilja V. Khavrutskii, Djamaladdin G. Musaev and Keiji Morokuma, et al. "Computational Insights into the Mechanism of Radical Generation in B12-Dependent Methylmalonyl-CoA Mutase." *J Am Chem Soc* 128 (2006): 1287-1292.

46. Li, Xin, Lung Wa Chung, Piotr Paneth and Keiji Morokuma. "DFT and Oniom (DFT: MM) Studies on Co– C Bond Cleavage and Hydrogen Transfer in B12-Dependent Methylmalonyl-CoA Mutase. Stepwise or Concerted Mechanism?." *J Am Chem Soc* 131 (2009): 5115-5125.

47. Neeraj, Kumar, Denis Bucher and Pawel M. Kozlowski. "Mechanistic Implications of Reductive Co–C Bond Cleavage in B12-Dependent Methylmalonyl CoA Mutase." *J Phys Chem B* 123 (2019): 2210-2216.

48. Neeraj, Kumar, Shubin Liu and Pawel M. Kozlowski. "Charge Separation Propensity of the Coenzyme B12–Tyrosine Complex in Adenosylcobalamin-Dependent Methylmalonyl–CoA Mutase Enzyme." *J Phys Chem Lett* 3 (2012): 1035-1038.

49. Kumar, Manoj and Pawel M. Kozlowski. "Role of Tyrosine Residue in the Activation of Co– C Bond in Coenzyme B12-Dependent Enzymes: Another Case of Proton-Coupled Electron Transfer?." *J Phys Chem B* 113 (2009): 9050-9054.

50. Pawel M, Kozlowski, Takashi Kamachi, Manoj Kumar and Kazunari Yoshizawa. "Initial Step of B 12-Dependent Enzymatic Catalysis: Energetic Implications Regarding Involvement of the One-Electron-Reduced Form of Adenosylcobalamin Cofactor." *J Biol Inorg Chem* 17 (2012): 293-300.

51. Conrad, Karen S, Christopher D. Jordan, Kenneth L. Brown and Thomas C. Brunold. "Spectroscopic and Computational Studies of Cobalamin Species with Variable Lower Axial Ligation: Implications for the Mechanism of Co–C Bond Activation by Class I Cobalamin-Dependent Isomerases." *Inorg Chem* 54 (2015): 3736-3747.

52. Brooks, Amanda J, Christel C. Fox, Marsh E, Neil G and Monica Vlasie, et al. "Electronic Structure Studies of the Adenosylcobalamin Cofactor in Glutamate Mutase." *Biochem* 44 (2005): 15167-15181.

53. Spataru, Tudor and Ronald L. Birke. "The Effect of Solvent on the Electrode Process of Methylcobalamine as Studied by Cyclic Voltammetry." *J Electroanal Chem* 593 (2006): 74-86.

54. Birke, Ronald L, Qingdong Huang, Tudor Spataru and David K. Gosser. "Electroreduction of a Series of Alkylcobalamins: Mechanism of Stepwise Reductive Cleavage of the Co– C Bond." *J Am Chem Soc* 128 (2006): 1922-1936.

55. Spataru, Tudor. "The Complete Electronic Structure and Mechanism of the Methionine Synthase Process as Determined by the MCSCF Method." *J Organomet Chem* 942 (2021): 121811.

56. Bersuker, Isaac B. "Limitations of Density Functional Theory in Application to Degenerate States." *J Comput Chem* 18 (1997): 260-267.

57. Spataru, Tudor and Francisco Fernandez. "The Nature of the Co-C Bond Cleavage Processes in Methylcob (II) Alamin and Adenosylcob (III) Alamin." *Chem J Mold* 11 (2016): 10-20.

58. Spataru, Tudor and Ronald L. Birke. "Carbon– Cobalt Bond Distance and Bond Cleavage in One-Electron Reduced Methylcobalamin: A Failure of the Conventional DFT Method." *J Phys Chem* A 110 (2006): 8599-8604.

59. Bersuker, Isaac B. Electronic Structure and Properties of Transition Metal Compounds: Introduction to the Theory. New York: Wiley-Interscience, USA, (1996).

60. Bersuker, Isaac B. "Pseudo-Jahn–Teller Effect A Two-State Paradigm in Formation, Deformation and Transformation of Molecular Systems and Solids." *Chem Rev* 113 (2013): 1351-1390.

61. Bersuker, Isaac B. "Jahn–Teller and Pseudo-Jahn–Teller Effects: From Particular Features to General Tools in Exploring Molecular and Solid State Properties." *Chem Rev* 121 (2020): 1463-1512.

62. Valiev, Marat, Eric J. Bylaska, Niranjan Govind and Karol Kowalski, et al. "NWChem: A Comprehensive and Scalable Open-Source Solution for Large Scale Molecular Simulations." *Comput Phys Commun* 181 (2010): 1477-1489.

63. Frisch, M. J, G. W. Trucks, H. B. Schlegel and G. E. Scuseria, et al. "Gaussian 16 Revision a. 03. 2016; Gaussian Inc." *Wallingford CT* 2 (2016).

64. García-Fernández, Pablo, Laura García-Canales, J. M. García-Lastra and Javier Junquera, M. Moreno, et al. " Pseudo-Jahn-Teller Origin of the Low Barrier Hydrogen Bond in N2H7+" *J Chem Phys*129 (2008): 124313.

65. Kohen, Amnon and Judith P. Klinman. "Hydrogen Tunneling in Biology." *Chemistry & biology* 6 (1999): R191-R198.

66. Lerner, T. Encyclopedia of Physics (2nd ed.). New York: VCH, USA, (1991).

67. Vrijbloed, Jan W, Katja Zerbe-Burkhardt, Ananda Ratnatilleke and Andreas Grubelnik-Leiser, et al. "Insertional Inactivation of Methylmalonyl Coenzyme A (CoA) Mutase and Isobutyryl-CoA Mutase Genes in Streptomyces Cinnamonensis: Influence on Polyketide Antibiotic Biosynthesis." *J Bacteriol* 181 (1999): 5600-5605.

68. Gorinchoy, Natalia, Iolanta Balan, Victor Polinger and Isaac Bersuker. "Pseudo Jahn-Teller Origin of the Proton-Transfer Energy Barrier in the Hydrogen-Bonded [FHF]-System." *Chem J Mold* 16 (2021): 115-120.

How to cite this article: Spataru, Tudor. "The Electronic Structure and Mechanism of the Adenosylcobalamin-Dependent Bio-Processes as Determined by the MCSCF Method." *Med Chem* 11 (2021): 595.