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A Combined Density Functional Theory and Molecular Mechanics Study of the Relationship between the Structure of Coenzyme B_{12} and Its Binding to Methylmalonyl-CoA Mutase

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Recent crystallographic studies of coenzyme B_{12}-dependent enzymes[1−6] have raised a series of questions related to the involvement of the axial base in the catalytic activation of the Co−C bond. In a certain subclass of B_{12} enzymes including methylmalonyl-CoA mutase (MCM)[1,2] or glutamate mutase (GLM),[3,4] the coenzyme is bound to the active sites of these proteins in conformation entirely different from that observed in the solution. The dimethylbenzimidazole (DBI) moiety is removed from the cobalt atom (∼10 Å) and replaced with a histidine from the protein. In other subclasses of B_{12} enzymes such as diol dehydratase (DD),[5,6] the conformation of coenzyme B_{12} bound to the active site resembles those observed in the solution. Furthermore, the Co−N_{His} bond length in MCM[1] is very long **(**2.53 Å**)**, and a similar Co−N_{DBI} bond length of 2.50 Å was also found for DD,[5] while a shorter bond length of 2.35 Å was reported for GLM.[3] In comparison the Co−N_{axial} bond lengths are 2.24 Å in coenzyme B_{12},[7] 2.19 Å in methylcob(III)alamin,[8] 2.09 Å in Coα-(1H-imidazolyl)-Coβ-methylcob(III)amide,[9] and 2.16 Å in cob(II)alamin,[10] respectively. The increase in the Co−N_{axial} bond length is significant and seems to be a key feature of these structures. It has been suggested that the crystallographically obtained "long" Co−N_{axial} bond is relevant for the activation of the protein-bound corrinoid cofactor toward homolysis of its Co−C bond[1] and that the ∼2.5 Å distance would stabilize the Co(II) species relative to Co(III), thus favoring adenosyl radical formation. However, the size of these enzymes, the inherently low crystallographic resolution, the mixed Co(III)/Co(II) states, and the nature of their upper axial ligands make interpretation of this structural data uncertain. In fact, comparison of the Co−N_{axial} lengths between organocob(III)alamins and cob(II)alamin does not indicate that a simple stretch of the Co−N_{axial} bond would lead to activation of the protein-bound cofactor toward homolysis.[11] The length of the Co−N_{axial} bond was also studied by EXAFS spectroscopy. In the case of MCM,[12] X-ray absorption spectroscopy has confirmed the presence of a "long" Co−N_{axial} bond, while for GLM[13] EXAFS data has indicated a "normal" axial cobalt−nitrogen bond. Whether the "long" Co−N_{axial} bond of the protein-bound corrinoid cofactor is real or an artifact[14] and if its lengthening has a functional implication for mechanism of the Co−C bond cleavage remain open questions.

While the problem of axial base influence on reactivity of cobalamins has been addressed extensively at the density functional theory (DFT)[15−21] and molecular mechanics (MM)[22,23] level, this work represents the first attempt to combined these approaches to elucidate the relationship between the structure of a free coenzyme and that bound to MCM. To understand how a protein environment affects the N_{His}−Co−C_{Ado} bond lengths, the essential components of the coenzyme B_{12}, including corrin, adenosyl group, as well as histidine bound to a protein chain were described by DFT, while the rest of the system was treated at a MM level. The combined DFT/MM calculations were performed using the GAUSSIAN 98[24]

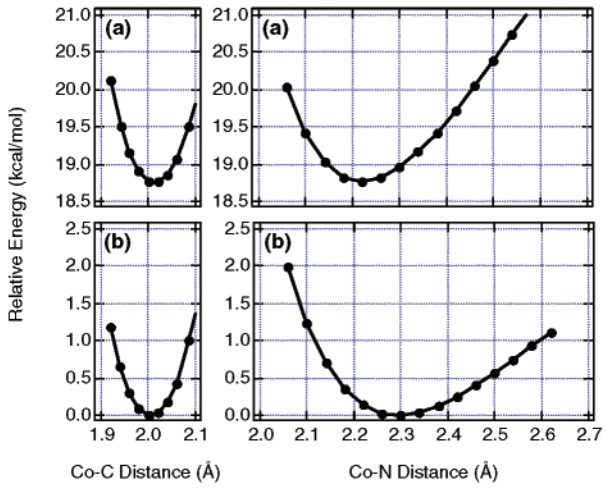

Figure 1. Interligand potential energy curves of His-[Co(III)corrin]-Ado associated with (a) free complex (b) complex embedded in protein. In the DFT/MM calculations the "atoms" belonging to the MM part were kept frozen.

and AMBER 6[25] programs, employing X-ray coordinates of a substrate-free MCM, deposited as 3REQ PDB file.[2] The B3LYP functional, successfully applied in previous calculations on cobalamins[15,17,19] with 6-31G(d) [for H,C,N,O] and Ahlrich's VTZ [for Co] basis sets, was employed in the present study. Upon the initial MM structure relaxation with respect to the location of hydrogen atoms,[26] the DFT optimization of a QM subunit, i.e., His-[Co(III)corrin]-Ado complex, was performed first in the gas phase, without interactions from the environment, and then in the presence of apoenzyme. In the combined DFT/MM calculations all atoms in the vicinity of the cobalt atom were treated quantum mechanically. The partition between the QM and MM subunits was performed outside His-Co-Ado moiety, employing *link atoms method*.[27,28] Two covalent bonds have been cut, one between His and the protein, and the second, associated with the nucleotide loop. In addition, the corrin was simplified with respect to the side chains.[29] Computational analysis showed that the inclusion of more atoms in the QM part associated with corrin substituents significantly increases computational cost but has negligible influence on interligand His-Co-Ado potential.

The comparison of these two optimized structures shows that the Co−C_{Ado} bond length, as well as structural parameters associated with corrin, were unaffected by the presence of apoenzyme. Only the Co−N_{His} bond length was slightly elongated from its equilibrium of 2.23 to 2.30 Å as shown in Figure 1, which also displays the results of the potential energy calculations for both interligand Co−X bonds (X = C_{Ado} or N_{His}).

The analysis of DFT/MM results show that the embedding of a His-[Co(III)corrin]-Ado complex to a protein matrix leaves the

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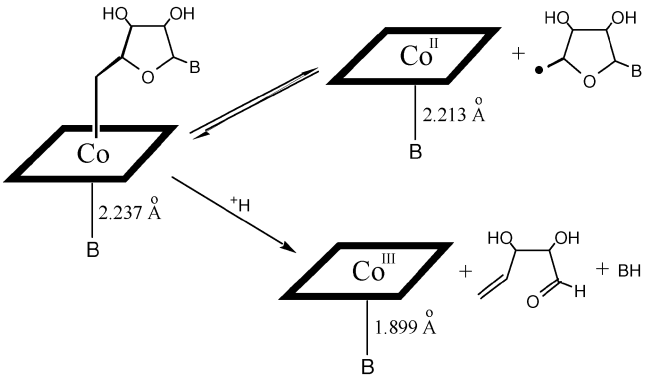

Figure 2. Changes in Co−N_B length corresponding to homolytic and heterolytic cleavage of the Co−C bond computed at B3LYP level of theory.

covalent Co−C_{Ado} bond intact (Figure 1a). This is consistent with resonance Raman data,[30] since the spectra of free coenzyme in the solution and bound to MCM are similar, and the ν_{Co-C} stretch is minimally perturbed in the bound state. In fact, noticeable weakening of the Co−C_{Ado} bond in the absence of substrate would be a disadvantage and is expected to lead to enzyme inactivation. The status of the cobalt−nitrogen axial base bond is quite different upon the embedding of complex to apoenzyme. The already weak Co−N_{His} bond is further elongated by 0.08 Å and noticeably labilized (Figure 1b). There are electrostatic and steric interactions between the QM and MM subunits, which can influence this interligand bond. However, the electrostatic component is dominant, and labilization of the Co−N_{His} bond that takes place in the bound state is mainly due to the electrostatic field exerted by the surrounding protein matrix. The fact that only the Co−N_{His} is influenced, but not Co−C_{Ado}, reflects the nature of N_{His}−Co−C_{Ado} axial binding which lies in an unusual combination of a poor σ/π donor of the axial base (His) and the unusually strong σ donor of the alkyl group (Ado).

The present DFT/MM calculations do not support the existence of a "long" Co−N_{axial} bond but rather predict a "normal" bond, which is labilized. The optimized Co−N_{His} distance of 2.30 Å is longer in comparison to a free cofactor (2.23 Å) but shorter than the crystallographically obtained value of 2.47 Å.[2] This difference, as well as inconsistency, between the reported Co−N_{axial} bond lengths obtained from X-ray crystallography,[1−5] EXAFS,[12,13] and EPR,[31] can be readily explained on energetic grounds: the elongation from 2.30 to 2.50 Å is not energetically very costly and worth only 0.5 kcal/mol (Figure 1b). This amount of energy can be easily exerted by the surrounding protein environment.

What possible implications can labilization of the Co−N_{axial} bond have on catalytic activation of the Co−C bond? To answer this, we considered two possible modes of the Co−C cleavage as shown in Figure 2. During enzymatic catalysis, the Co−C bond of coenzyme B_{12} is cleaved homolytically, leading to the formation of the 5′-deoxyadenosyl radical and cob(II)alamin.[32] The Co−N_{DBI} bond length of 2.16 Å in cob(II)alamin[10] is slightly shorter in comparison to that of the coenzyme, which is 2.24 Å.[7] Computational studies using gradient-corrected DFT have confirmed these crystallographic findings and have shown that the axial base has only minor influence on Co−C bond cleavage in cobalamins.[17−21] In the case of the heterolytic cleavage, which is an abiological side reaction, this change is predicted to be significant. Because the X-ray data is not available for the corrinoid product of heterolysis, the DFT calculations estimated that the length of this bond should be around 1.9 Å. The heterolysis of the Co−C bond, which involves an internal fragmentation of the Ado group, is minimized as a nonproductive side reaction during enzymatic catalysis.[33,34] The significant difference in the Co−N_{axial} bond lengths between homolysis and heterolysis products (Figure 2), indicates that control of the Co−N_{axial} bond length may be important primarily to inhibit the Co−C heterolysis. This is consistent with the proposal[35] that control of this distance is critical in the biological processes and is also supported by current DFT calculations. Thus, one may conclude that the labilization of the Co−N_{axial} bond, which takes place in the coenzyme B_{12}-dependent enzymes, is most likely necessary for the fine-tuning of the cobalt−nitrogen(axial base) distance. The controlling of this distance is important to inhibit abiological side reactions involving heterolysis of the Co−C bond but is not important for biologically relevant Co−C bond homolysis.

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