Resonance Raman Spectra Show That Coenzyme B_{12} Binding to Methylmalonyl-Coenzyme A Mutase Changes the Corrin Ring Conformation but Leaves the Co−C Bond Essentially Unaffected

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We report the first resonance Raman (RR) spectra of coenzyme B_{12} in an active B_{12}-dependent enzyme, methylmalonyl coenzyme A (MMCoA) mutase. The spectra reveal a large change in the corrin ring conformation, consistent with X-ray crystallography.[1] However, isotope labeling reveals the Co−C force constant to be only slightly altered, despite the dramatic activation of this bond in the catalytic cycle of this enzyme.[2]

The Co−C bond is found in two enzyme cofactors, methylcobalamin (MeCbl) and 5′-deoxyadenosylcobablamin (AdoCbl).[3] The enzymes utilizing MeCbl catalyze methyl transfer reactions,[4] while those employing coenzyme B_{12} (or AdoCbl, Figure 1) catalyze rearrangement reactions.[5] Among the latter, MMCoA mutase is the only isomerase found in both bacteria and mammals. It catalyzes the conversion of methylmalonyl coenzyme A to succinyl coenzyme A (Scheme 1).

The rearrangement reaction is initiated by homolytic cleavage of the Co−C bond, forming an adenosyl radical and cob(II)alamin.[5] The homolytic Co−C cleavage rate is dramatically enhanced in B_{12}-dependent enzymes. Upon substrate binding to the enzyme, the rate of appearance of Co(II) is 60 s^{-1} for diol dehydrase,[6] $\geq$300 s^{-1} in ethanolamine ammonia-lyase,[7] and $\geq$600 s^{-1} for MMCoA mutase,[8] compared to 4×10^{-10} s^{-1} for AdoCbl in aqueous solution at 25 °C.[9] This $\sim 10^{12\pm1}$ rate enhancement of Co−C bond homolytic cleavage amounts to an ~15.5 kcal/mol destabilization of the Co−C bond.[9] However, the Co−C stretching frequency for AdoCbl is nearly as high (424 cm^{-1}) in MMCoA mutase as it is in aqueous solution (430 cm^{-1}) (Figure 2).

To minimize photolysis, the RR spectra were obtained on frozen samples by laser excitation at 568.2 nm, on the low energy side of the complex AdoCbl absorption spectrum (Figure 3). The absorption bands arise from multiple $\pi-\pi^*$ electronic transitions of the corrin ring,[10] and corrin vibrational modes dominate the RR spectra.[11,12] Nevertheless, the Co−C stretch (ν_{Co-C}) is enhanced,[13] albeit weakly, along with a deformation mode of the adenosyl ribose ring (δ_{ribose}) at 568 cm^{-1},[14] which involves motion of the Co-bound C5′ atom (Figure 1). These bands are readily detected by ^{13}C5′ substitution (Figure 2).

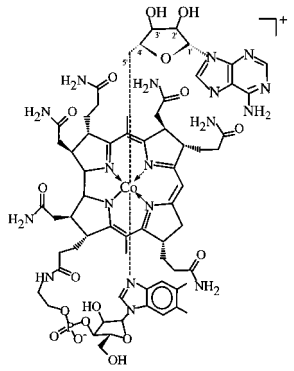

Figure 1. Structure of coenzyme B_{12}, AdoCbl.

Scheme 1. Isomerization of MMCoA Catalyzed by MMCoA Mutase

While the δ_{ribose} mode is unaffected by enzyme binding, and ν_{Co-C} is shifted only 6 cm^{-1}, some of the corrin RR bands display major changes in either frequency or intensity. These changes are evident in both the low-frequency (Figure 2) and high-frequency (Figure 4) regions, implying a change in the corrin conformation. We note that the RR changes are much larger than could be suggested by the relatively minor shape change of the absorption spectrum (Figure 3). Moreover the same RR changes are seen for the hydroxocobalamin product of photolysis (data not shown) although the absorption spectra (Figure 3, bottom) are again very similar for the free and enzyme-bound cofactor. Although quantitative interpretation will require a normal coordinate analysis, it is significant that all the modes above 1480 cm^{-1} shift up, by 3−11 cm^{-1} in the spectrum. We interpret this pattern as resulting from a flattening of the macrocycle, a trend that has been documented in metalloporphyrins.[15] The crystal structure of MMCoA mutase[1] does indeed reveal a flattening of the corrin relative to its strongly ruffled conformation in enzyme-free AdoCbl.[16] The highest frequency RR band, at ~1600 cm^{-1}, is especially sensitive, and it probably arises from a mode involving out-of-phase stretching of adjoining meso-bridge bonds, similar to ν_{10} or ν_{19} in porphyrins,[15] which are also especially sensitive to conformation. For such a mode, the frequency upshift on flattening results from enhanced kinematic interaction of the adjoining bonds.

Because of the steric crowding in AdoCbl, an enzyme induced change in the corrin conformation could weaken the Co−C bond via nonbonded forces. The effect of such forces is illustrated by the dramatically higher ν_{Co-C}, 506 cm^{-1}, when the bulky adenosyl group is replaced by a methyl group, in MeCbl.[11,12] The force

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(13) The 440/430 cm^{-1} pair of peaks observed in the difference spectrum between natural abundance AdoCbl and 5′-CD_2 AdoCbl in refs 11 and 12 are not observed with 5′-^{13}C labeled AdoCbl. Instead, only the 430 cm^{-1} peak is observed. Therefore, the 440 cm^{-1} peak does not represent the ν_{Co-C} of an alternate conformer, but probably is due to a strong corrin ring mode coupled to 5′-CH_2.
(14) This mode is upshifted to 600 cm^{-1} in 2′, 5′-dideoxyadenosylcobalamin in which the 2′-OH group is substituted by a hydrogen atom. Dong, S.; Padmakumar, R.; Banerjee, R.; Spiro, T. G. Munuscript in preparation.
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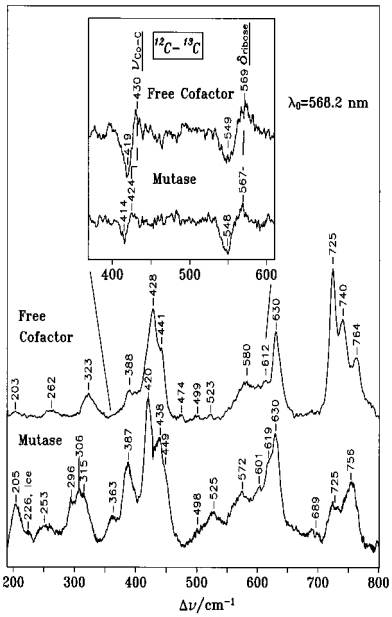

Figure 2. Low-frequency RR spectra of coenzyme B_{12} in aqueous solution and in the enzyme. The insets are difference spectra obtained with 5′-^{13}C-AdoCbl and identify the ν_{Co-C} and δ_{ribose} vibrations. The bacterial MMCoA mutase was overexpressed in *E. coli* strain K38 and purified as previously described.[21] The recombinant expression vector (pMEX2/pGP1-2) harboring the *Propionibacterium shermanii* genes was a gift from Dr. P. Leadley (Cambridge University). 5′-^{13}C-AdoCbl was a gift from Dr. Kenneth L. Brown (Ohio University). Isotopically labeled holoenzymes were prepared by reconstitution with the corresponding isotopically labeled cofactors according to published procedures.[22] Free cofactors were removed from the holoprotein by HPLC purification. Protein solutions for Raman studies were concentrated to 0.6−1.3 mM in coenzyme B_{12}. The 20 μL samples were loaded into sample cells in a liquid nitrogen dewar[22] under reduced light at 4 °C in a glovebag purged with nitrogen. The dewar was then pumped to prevent frost formation on the surface of the sample. Resonance Raman spectra were collected on a Spex 1877 triple monochromator equipped with a intensified photodiode array as the detector. Excitation laser lines were from a Kr^+ laser (Coherent Innova 100-K3A). The following conditions were used for all the experiments: excitation wavelength = 568.2 nm, laser power at the sample = 20 mW, spectral slit width = 3 cm^{-1}, scattering geometry = ∼135° backscattering. Raman shifts were calibrated with known solvent spectra. Spectral analysis and baseline correction were carried out with Labcalc (Galactic). To precisely compare different isotopically labeled cofactors or proteins, the samples to be compared were always loaded side by side on the liquid nitrogen dewar; their spectra were collected with the same instrumental settings. Difference spectra were obtained by normalizing the two spectra with the 630 cm^{-1} peak as reference.

constants in MeCbl and AdoCbl are directly proportional to the activation energies for Co−C homolysis, 37 ± 3 kcal/mol[17] and 30 ± 2 kcal/mol,[9] and establish that the bond weakening in AdoCbl is a ground-state effect. However, the additional ν_{Co-C} shift induced by MMCoA mutase translates to only ∼0.5 kcal/mol additional Co−C weakening, a small fraction of the ∼15 kcal enzymatic activation.[9] We note that this result is consistent with the negligible change in Co−C bond distance (1.97 ± 0.05 and 1.98 ± 0.05 Å for mutase bound and free AdoCbl, respectively) revealed by EXAFS.[18]

If a steric mechanism is employed by the enzyme, then most of the effect must be expressed when the substrate binds. Thus the enzyme cofactor interaction would be a prelude to further conformation change upon substrate binding. However, it is also possible that the activation results from transition state stabilization instead of ground-state destabilization. The causal factor in that case would not be enhanced steric repulsion, but rather enhanced stabilization of the cob(II)alamin. Such an effect is seen when base-off AdoCbl is compared with base-on AdoCbl.[11,12] The ν_{Co-C} frequency is unaffected, but the homolysis activation energy decreases by ∼4 kcal/mol,[19,2] reflecting cob(II)alamin stabilization by the axial base. A similar mechanism is possible in MMCoA mutase, whose crystal structure reveals the benzylimidazole to be displaced by a histidine residue.[1] Although the nature of the axial base does not affect the Co−C force constant,[20] increasing basicity is expected to help stabilize cob(II)alamin.

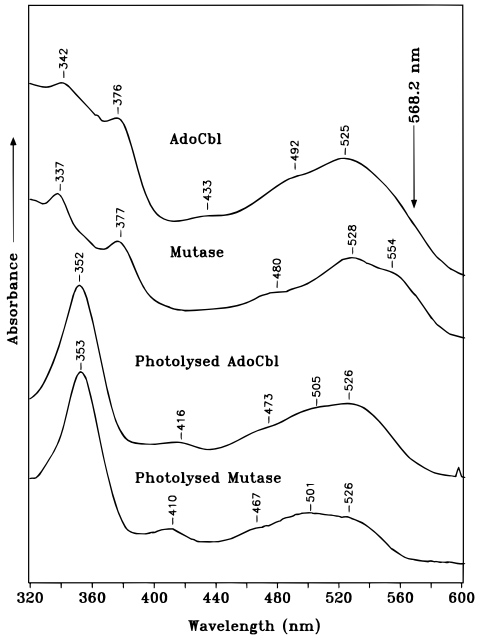

Figure 3. Visible absorption spectra of intact (top) and aerobically photolyzed (bottom) coenzyme B_{12}, in aqueous solution and bound to the mutase (∼0.08 mM in 50 mM potassium phosphate buffer, pH 7.5).

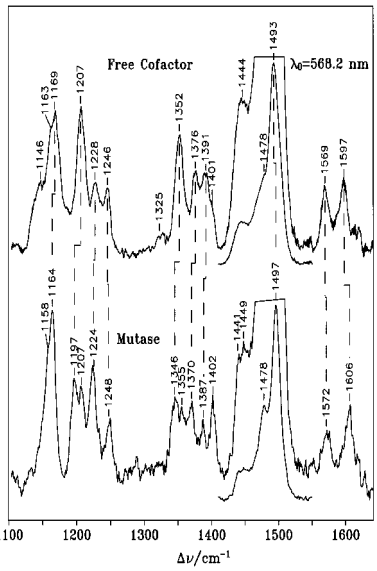

Figure 4. High-frequency RR spectra of coenzyme B_{12} in aqueous solution and in the enzyme. Experimental conditions as in Figure 2.

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