

Near-IR Fourier Transform Raman Spectroscopy of Photolabile Organocobalt B₁₂ and Model Compounds. Identification of the Co–C Bond Stretch in Cobalamins

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The frequency of the Raman-active Co–C bond stretch in photolabile methylcoenzyme B₁₂ has been conclusively determined by the new technique of near-IR Fourier transform (FT) Raman spectroscopy to be 500 cm⁻¹ for methylcobalamin and 470 cm⁻¹ for deuteriated methyl (CD₃) cobalamin.

The unrecognized photolability of the B₁₂ coenzymes led to the initial isolation of the more stable cyanocobalamin, now known as vitamin B₁₂.¹ Subsequent structural elucidation of coenzyme B₁₂ (adenosylcobalamin) revealed that the natural cofactor contains an unprecedented Co–C bond to the axial substituent, 5'-deoxyadenosyl.² Although thermostable, the Co–C bond is homolytically cleaved by B₁₂-dependent enzymes as an essential step in B₁₂-dependent catalysis.^{1,3–6}

A direct assessment of the process whereby the Co–C bond is cleaved has been hampered by both the large size of the B₁₂ holoenzymes and the photosensitivity of the coenzyme. No X-ray or detailed NMR characterization has been reported for a B₁₂ holoenzyme. Although structural results are available for several organocobalamins,^{2,7,8} including the cofactors adenosylcobalamin² and methylcobalamin,⁷ the relatively

large size of these cofactors limits the precision of the structural parameters.

Vibrational spectroscopy has not been widely applied to B₁₂ biochemistry. Previous resonance Raman studies were hindered by sample photodecomposition, and vibrational modes associated with the Co–C moiety or axial ligands were not detected.^{9,10} With the advent of near-IR-excited Fourier transform (FT) Raman spectroscopy, we have begun to examine photolabile organocobalt compounds.^{11,12} The anticipation that near-IR laser excitation at 1.064 μm would eliminate Co–C photolysis and fluorescence interference previously encountered with VIS/near-UV excitations has been amply demonstrated for photolabile organocobalt B₁₂ models.^{11,12} In this report, we extend our study to biologically active cobalamins.

Figure 1 shows the FT Raman spectra of CH₃-cobalamin and CD₃-cobalamin in their crystalline state. The strong Raman line at 500 cm⁻¹ is isotopically sensitive, and its 30 cm⁻¹ down-shift upon deuteration of the axial methyl is

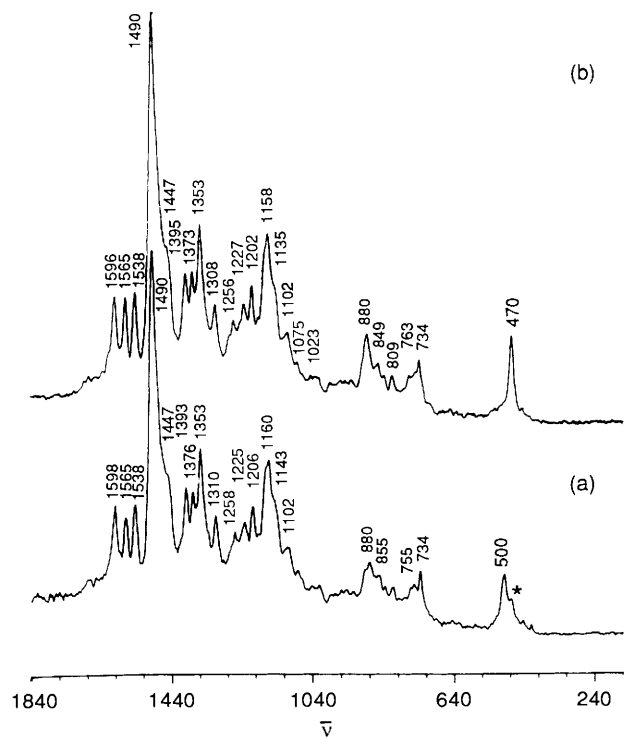


Figure 1. FT Raman spectra of methylcobalamin and deuteriated methyl (CD₃) cobalamin obtained in the solid state. Conditions: excitation wavelength, 1.064 μm; laser power, 1.0 W; acquisition time, 8 min; spectral resolution, 4.0 cm⁻¹. The FT Raman spectra were recorded by using a Bomem DA3.02 spectrophotometer equipped with a liquid nitrogen cooled InGaAs detector. Near-IR excitation was provided with a Quantronix CW Nd:YAG laser. Scattered Raman photons were collected at 180° using an ellipsoidal mirror, and the Rayleigh line was removed through the use of three dielectric filters. (a) Methylcobalamin, (b) CD₃-cobalamin. (*) Spurious line.

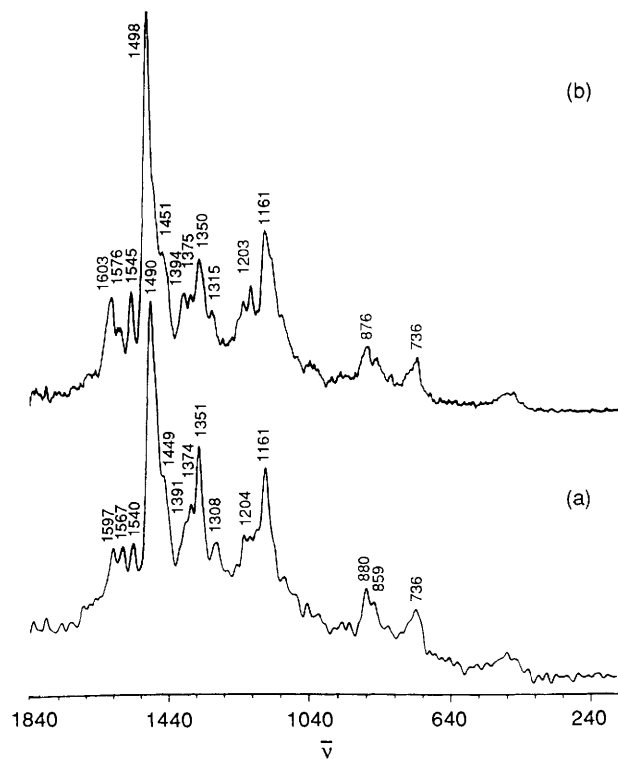


Figure 2. FT Raman spectra of adenosylcobalamin and cyanocobalamin in the solid state. Data acquisition conditions were the same as in Figure 1. (a) Adenosylcobalamin, (b) cyanocobalamin.

identical to the isotopic shift of the Co-CH₃ stretching mode observed in the model compounds,¹¹ pyCo(DH)₂CH₃ and pyCo(DH)₂CD₃ (where py = pyridine and DH = monoanion of dimethylglyoxime). This line is thus unequivocally identified as the Raman-active Co-CH₃ stretching mode in biologically active methylcobalamin. The observed frequency of 500 cm⁻¹ is well within the range (522–480 cm⁻¹ depending on L) found in B₁₂ models of the type, LCo(DH)₂CH₃,¹² where L = neutral axial ligand. The similar values of the Co-C stretching frequencies for the cofactor and the models are consistent with the X-ray structural data, which show nearly identical Co-CH₃ bond lengths of ~2.0 Å.³

We have also investigated the influence of crystal structures on the Co-C bond stretch by varying the recrystallization conditions. The same Raman line at 500 cm⁻¹, however, is detected for the less crystalline commercial sample (Sigma) and methylcobalamin recrystallized in aqueous acetone, aqueous 2-methylpentane-2,4-diol, aqueous t-butanol, or ammonium sulphate solution. The Co-C stretching frequency in cobalamin crystals thus appears to be insensitive to the environment. On the other hand, recrystallization does influence the equatorial corrin vibrations, as judged by significant differences in Raman line frequencies and relative intensities between less crystalline and recrystallized methylcobalamin.

In Figure 2 the FT Raman spectra of adenosylcobalamin and cyanocobalamin obtained in the solid state are compared. It is noted that the characteristically intense and sharp Raman line of the Co-CH₃ bond stretch is absent in the FT Raman spectrum of adenosylcobalamin. As compared to the axial methyl group, the greater mass of the axial adenosyl ligand lowers the ν(Co-C) frequency, and its structural complexity is likely to lead to increased coupling of the Co-C stretching mode with other vibrations. Conclusive identification of the ν(Co-C) frequency must await isotopic data. In the cyanocobalamin spectrum, a moderately intense line at 2130 cm⁻¹ can be assigned to the Raman-active cyano stretching mode, on the basis of the IR cyano stretching frequency¹³ at 2132 cm⁻¹ and the absence of such a line in the FT Raman spectra of the other cobalamins.

In contrast to resonance Raman studies,^{9,10} our FT Raman results indicate that the nature of the sixth axial ligand profoundly influences the corrin vibrational frequencies. Briefly, the corrin vibrations for adenosylcobalamin, Figure 2(a), are noticeably shifted to higher frequencies for cyanocobalamin, Figure 2(b). Understandably, replacement of an alkyl group with CN alters the electron density around the Co atom, which in turn influences the vibrational properties of the equatorial macrocycle. Similar corrin vibrational frequencies are observed for the two cofactors, reflecting the presence of a strongly electron donating alkyl group in both molecules.

Our near-IR FT Raman results indicate that the Co-C bond stretch in B₁₂ model compounds¹² is nearly invariant compared to the Co-C bond dissociation energy (BDE, or perhaps

more correctly, bond disruption enthalpy¹⁴), suggesting that the BDE differences arise from a transition state effect and/or factors distributed throughout the molecule. This interpretation can be extended to cobalamins since the Co-C stretching frequencies of pyCo(DH)₂CH₃ and methylcobalamin are similar (~500 cm⁻¹), but the measured BDEs are 33 kcal mol⁻¹ (138 kJ mol⁻¹)¹⁵ and 37 kcal mol⁻¹ (155 kJ mol⁻¹),¹⁶ respectively. Our demonstration that high quality FT Raman spectra are readily obtained for biologically active cobalamins opens up the possibility of directly measuring the Co-C bond strength in B₁₂-holoenzymes.

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