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Review

Excited electronic states and internal conversion in cyanocobalamin

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ABSTRACT

Cyanocobalamin (CNCbl) is a paradigm system for the study of excited electronic states and biological cofactors including the B_{12} vitamers. The photophysics of CNCbl has been thoroughly investigated using both ultrafast spectroscopy and time dependent density functional theory (TD-DFT). Here we review the spectroscopic and theoretical investigations of CNCbl with emphasis on the nature of S_1, the lowest excited electronic state, and extend the spectroscopic measurements to include the ultraviolet region of the spectrum. Ultrafast transient absorption measurements in the visible $\alpha\beta$ band region and in the mid-infrared led to assignment of the S_1 state to a ligand-to-metal charge transfer (LMCT) with lengthened axial bonds and a ~3 kcal/mol barrier for internal conversion to the ground state. The present measurements encompassing the γ band region of the spectrum provide further support for the assignment of the S_1 state. The experiments are in good agreement with the results of TD-DFT calculations which confirm the expected lengthening of the axial bonds in S_1 and account for the observed barrier for internal conversion back to the ground state.

1. Introduction

Vitamin B_{12} (cyanocobalamin, CNCbl) and its derivatives are essential human nutrients, originally discovered as a treatment for pernicious anemia [1–5]. The B_{12} vitamers (a vitamer is one of a class of related chemical substances that fulfill the same specific vitamin function) are cobalt centered corrinoid rings featuring four equatorial nitrogen ligands provided by the ring along with variable upper and lower axial ligands. The electronic structure and chemical function of cobalamin compounds are controlled by the axial ligands. In solution and in some enzymes, for example ethanolamine deaminase [6], the lower axial ligand is a dimethylbenzimidazole group attached to the corrin ring through an alkylphosphate tail (Fig. 1). In a number of other enzymes, including the human enzymes methylmalonyl Co-A mutase [7] and methionine synthase [8], the dimethylbenzimidazole ligand is replaced by a histidine residue. The lower axial ligand can also be replaced by a water molecule resulting in a base-off cobalamin compound. This base-off form dominates in solution at low pH [9], in certain transport proteins [10] and a small number of methyl transferase proteins [2]. The upper axial ligand is an alkyl group in the enzymatically active B_{12} coenzymes. Methylcobalamin (MeCbl) catalyzes methyl transfer reactions in methionine synthase while 5′-deoxyadenosylcobalamin (AdoCbl) catalyzes radical rearrangement reactions in a variety of enzymes including human methylmalonyl Co-A mutase [5].

In addition to the native biological function, cobalamin cofactors have recently received increased attention as a vehicle for optically controlled drug delivery and as a source of hydroxyl radicals [11,12] and as antivitamins [13,14]. The effective development of photoactive cobalamin compounds requires a detailed understanding of the intramolecular and intermolecular factors that control their photochemistry and photophysics. It is helpful to begin with the simplest cofactors and progress through to the more complex cobalamin compounds. Here we review the current state of understanding arising from ultrafast spectroscopic studies and theoretical simulations for the simplest non-alkylcobalamin CNCbl and present new broadband transient absorption measurements on CNCbl as a function of solvent.

2. Background

The electronic structure and chemical function of cobalamin compounds are controlled by the axial ligands. Ultrafast broadband transient absorption spectroscopy has been used to study the photochemistry and photophysics of naturally occurring and

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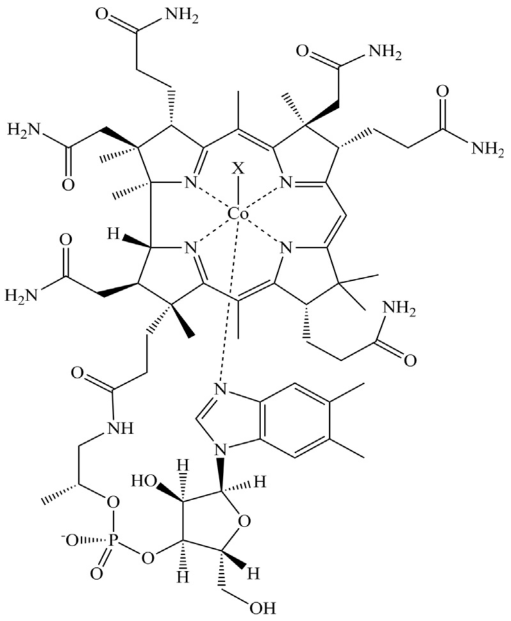

Fig. 1. Cobalamin cofactor. The upper axial ligand, designated X, can be an alkyl or non-alkyl group as discussed in the text.

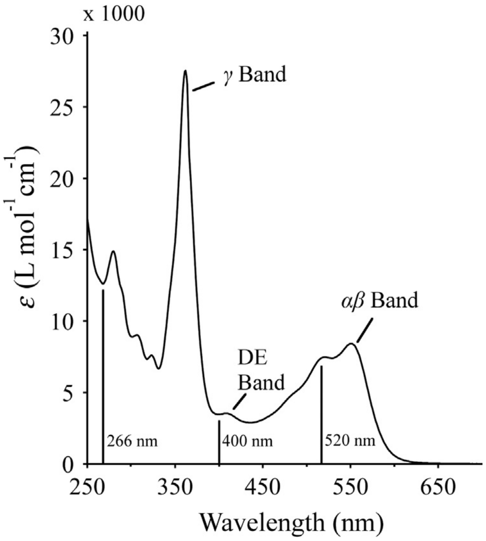

Fig. 2. Absorption spectrum of CNCbl in water. The vertical lines at 266 nm, 400 nm, and 520 nm indicate excitation wavelengths used in ultrafast spectroscopic measurements.

synthetically prepared cobalamin compounds [9,15–27]. Alkylcobalamins are photolabile. For most alkylcobalamins that have been studied to date, the primary photolysis quantum yield is on the order of unity [15,21,22,24]. The quantum yield for long-lived radicals is determined by competition between diffusive separation of the radical pair and geminate recombination [15]. Methylcobalamin is an exception with a pronounced wavelength dependence to the primary photolysis quantum yield [19,23,25]. Derivatives containing non-alkyl upper axial ligands (such as cyano-, hydroxo-, aquo-, and azido-) present markedly different photochemistry [16,17,26]. The photoexcitation of non-alkyl cobalamins is characterized by formation of short-lived electronically excited states that recover to the ground state on a picosecond timescale with little to no photolysis quantum yield.

Time dependent density functional theory (TD-DFT) studies by Kozlowski and coworkers have identified a triplet sigma antibonding state that was suggested to mediate the photolysis of alkyl cobalamins [28,30–34]. Detailed comparison of MeCbl and ethylcobalamin (EtCbl) led to the suggestion that changes in the barrier for crossing from the S_1 state to the dissociative channel accounted for the distinctive wavelength dependence and photolysis quantum yield following excitation of MeCbl. A more recent calculation exploring the S_1 potential energy surface has called this into question, suggesting instead that a ligand field state plays the crucial role in controlling the photodissociation [29]. In the non-alkyl cobalamins, the dissociation channel is much higher in energy and shifted far from the Franck–Condon region and thus is a non-relevant excited state pathway [30,35–38].

3. The S_1 state of CNCbl

Vitamin B_{12}, CNCbl, provides a paradigm system for the investigation of nonalkyl cobalamins. Careful transient absorption measurements combined with detailed theoretical simulations have illuminated many of the details of the electronic structure controlling the photochemistry and photophysics.

Ultrafast spectroscopic methods including broadband visible transient absorption spectroscopy and infrared spectroscopy have been used to study the photochemistry and photophysics of CNCbl after excitation of the DE and $\alpha\beta$ bands (Fig. 2) [16,17]. The bandwidth of the probe in the broadband visible transient absorption experiments ranged from *ca.* 450–700 nm, allowing global kinetic analysis of the decay of the excited state absorption and recovery of the $\alpha\beta$ band ground state bleach. The dynamics of the excited state absorption are characterized by one or two subpicosecond components followed by a solvent-dependent *ca.* 10 ps decay of the transient absorption signal [16,17,26]. The subpicosecond components account for rapid internal conversion from the initially excited electronic state to the lowest energy excited state, S_1. The solvent-dependent picosecond lifetime of the S_1 state reflects the influence of a small barrier along the pathway for internal conversion from the S_1 state to the ground state.

The ultrafast electronic relaxation of CNCbl has also been measured for the gas phase isolated molecule using excitation at 400 nm followed by 800 nm multiphoton ionization or excitation at 266 nm followed by 400 nm multiphoton ionization [27]. The signals obtained in both measurements are characterized by a strong ionization signal at early times. Following excitation at 400 nm this signal decays to baseline on a 100 ± 30 fs time scale. Following excitation at 266 nm the ionization signal decays on a 100 ± 10 fs time scale with an additional small amplitude signal (5%) decaying on a longer picosecond time scale. The data reflects fast internal conversion from the initially excited state to a relaxed state, presumably the S_1 state. This fast internal conversion is consistent with the time scales observed in solution. The low intensity of the picosecond evolution is attributed to the nature of the experiment. Ionization of the S_1 state will require an additional photon.

Time-resolved IR (TRIR) measurements probing the C≡N triple bond in ethanol are characterized by a significant red-shift of the C≡N stretching vibration from 2138 cm^{-1} in the ground state to 2120 cm^{-1} in the S_1 state [17]. This frequency is significantly higher than CN$^-$ at 2080 cm^{-1} consistent with a C–Co bond that is weakened, but not broken, in the S_1 excited state. The visible absorption of the S_1 state between 460 nm and 640 nm is characterized by a blue shift of the $\alpha\beta$ band similar to that observed for base-off alkylcobalamins [17]. A blue shift of the $\alpha\beta$ band is also observed for cyanocobinamide [39]. Thus the infrared absorption suggests a weakening of the C–Co bond and the shift in the electronic absorption spectrum is consistent with a weakening of the axial Co–N bond. As a result the S_1 state was tentatively assigned as a $\pi \rightarrow \sigma*(d_{z^2})$ ligand-to-metal charge transfer (LMCT) state.