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COMMUNICATION

Excited state electron transfer after visible light absorption by the Co(I) state of Vitamin B$_{12}$

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**The first example of excited state electron transfer from cob(I)alamin is reported herein. Vitamin B$_{12}$ was anchored to a mesoporous TiO$_2$ thin film and electrochemically reduced to the cob(I)alamin form. Pulsed laser excitation resulted in rapid excited state electron transfer, $k_{et} > 10^8$ s^{-1}, followed by microsecond interfacial charge recombination to re-form cob(I)alamin. The supernucleophilic cob(I)alamin was found to be a potent photoreductant. The yield of excited state electron transfer was found to be excitation wavelength dependent. The implications of this dependence are discussed.**

The ubiquitous cofactor cobalamin has been the subject of extensive research due to its unusual ability to exist in highly reduced states and form organometallic intermediates in aerobic biological systems.[1–8] Cobalamin and cobamide dependent enzymes perform a diverse array of difficult reactions that proceed *via* both radical and non-radical pathways including methyl transfer, rearrangement, and dehydration.[9,10] We report the first observation of electron transfer from the excited state of cob(I)alamin after visible light excitation.

A thin film (~10 μm) of nanocrystalline, mesoporous anatase titania was deposited on a transparent conductive glass (fluorine-doped tin oxide, FTO) and sintered at 420 ºC for 30 minutes. Cyanocobalamin (vitamin B$_{12}$) or dicyanocobyrinic acid heptamethyl ester (a B$_{12}$ derivative herein referred to as cobyrinate) was anchored to the thin film by soaking in a dilute acetonitrile solution of the chromophore to form an electrode hereafter referred to as TiO$_2$|Cob(III) (see cartoon in Fig. 1 inset). In a standard 3 electrode cell under argon atmosphere, the TiO$_2$|Cob(III) working electrode was immersed in a 100 mM tetrabutylammonium perchlorate acetonitrile solution with a Pt mesh counter electrode and a Ag/AgCl reference electrode. A forward bias was applied potentiostatically to sequentially reduce TiO$_2$|Cob(III) to TiO$_2$|Cob(II) and TiO$_2$|Cob(I). The identity of the reduced species was verified by electronic absorbance spectra, Fig.

1. The absorbance profiles closely matched those generated by chemical reduction in solution (Fig. S1) and were consistent with literature spectra.[11] The absorbance of the TiO$_2$|Cobyrinate in the Co(I) and Co(III) oxidation states are very similar, and can be found in Fig. S2.
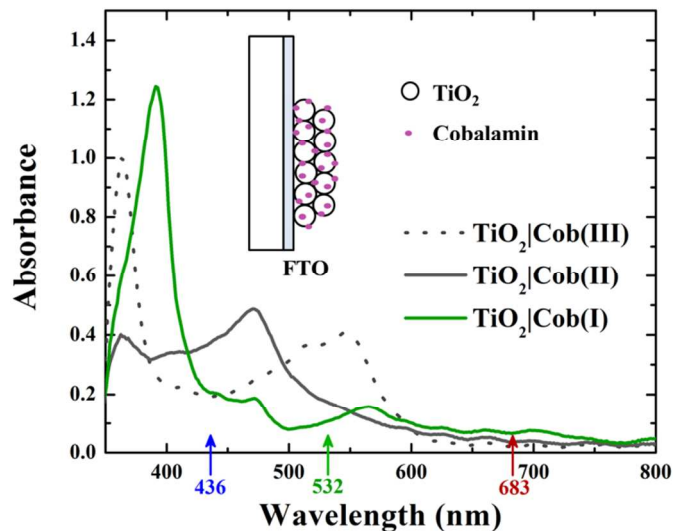

Fig. 1 The absorbance of cobalamin anchored to TiO$_2$ in 100 mM tetrabutylammonium perchlorate CH$_3$CN in the cyanocob(III)alamin (grey dotted), cob(II)alamin (grey solid), and cob(I)alamin (green) oxidation states. The inset shows a cartoon of the sample: FTO coated glass with a thin (~10 μm) film of TiO$_2$ nanoparticles (~20 nm diameter), to which the cyanocobalamin was anchored. The colored arrows along the horizontal axis indicate the excitation wavelengths utilized in transient absorption experiments.

In its reduced state, TiO$_2$|Cob(I) was irradiated with pulsed laser excitation at 1 Hz and the time-resolved change in absorbance was monitored using a previously described apparatus[12] (cobalamin shown in Fig. 2). There was a decrease in absorbance centered at ~390 nm and a relatively small increase in absorbance after the ~416 nm isosbestic point between TiO$_2$|Cob(I) and TiO$_2$|Cob(II), Fig. 1. The overlaid solid line in Fig. 2 is the scaled difference between the authentic spectra of TiO$_2$|Cob(II) and TiO$_2$|Cob(I). This simulation provides an excellent match to the observed spectral data, confirming that the electron transfer reaction in Eq. 1 occurred within the instrument response time, $k_{et} > 10^8$ s^{-1}.

$$TiO_2|Cob(I) + hv \rightarrow TiO_2(e^-)|Cob(II) \quad\quad (1)$$

$$TiO_2(e^-)|Cob(II) \rightarrow TiO_2|Cob(I) \quad\quad (2)$$
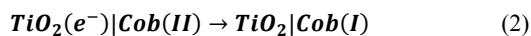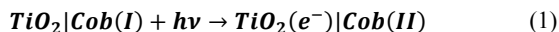
Cob(II) was reduced to Cob(I) on a micro- to millisecond timescale *via* interfacial charge recombination (Eq. 2) with maintenance of isosbestic points, implying a conversion between only these two states.
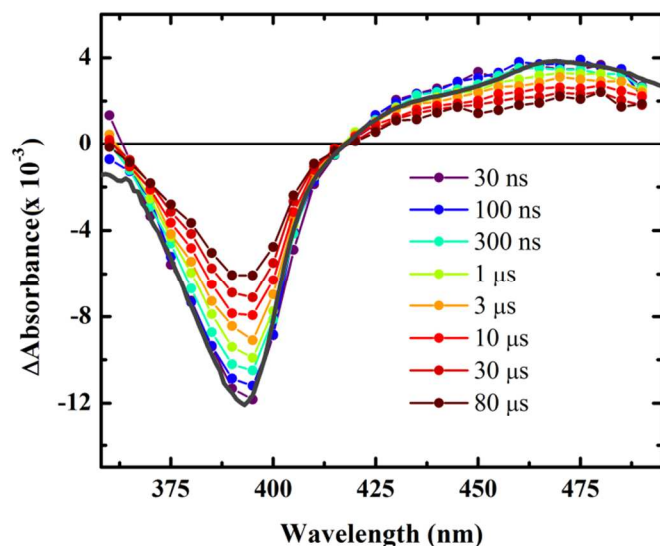
Fig. 2 The change in absorbance of TiO$_2$|Cob(I) in 100 mM tetrabutylammonium perchlorate CH$_3$CN after 532 nm pulsed laser excitation. The full-width at half maximum of the excitation beam was ~10 ns. The dark grey line overlaid is the simulated difference spectrum using the scaled difference between TiO$_2$|Cob(II) and TiO$_2$|Cob(I).

The charge recombination (Eq. 2) kinetics were non-exponential, but were well modeled by a stretched exponential function to obtain a pseudo first-order rate constant as described in the ESI.[13–17] Similar behavior was observed for the cobyrinate (Fig. S3). It was found that the rate constant for Eq. 2 was exponentially dependent upon the potentiostatically controlled quasi-Fermi level and spanned from 10^3 – 10^7 s^{-1} for the applied potential range (Fig. S4, S5). These observations are consistent with previously reported interfacial recombination.[18] The exact lifetimes and corresponding transient absorption data can be found in the ESI.

The transient absorption spectra shown in Fig. 2 were measured after 532 nm excitation, but can be taken as representative as the transient absorbance spectra after 532, 436, and 683 nm excitation were found to be normalizable within experimental error. The excitation

wavelengths are indicated by arrows on the horizontal axis of Fig. 1. The relative quantum yield of excited state electron transfer was quantified by transient absorption 20 ns after excitation. The concentration of the Co(II) compound formed was quantified and was divided by the number of absorbed photons at the excitation wavelength. This was repeated at all three excitation wavelengths and the highest yield was assigned a value of unity (Table 1).

Table 1 The relative quantum yields and reduction potentials of TiO$_2$|Cobalamin and TiO$_2$|Cobyrinate measured in 100 mM tetrabutyl ammonium perchlorate CH$_3$CN.

	V *vs.* NHE	Φ_{rel} (± 0.05)		
Complex	Co(II/I)	436 nm	532 nm	683 nm
TiO$_2$|Cobalamin	-0.63	0.15	1	0.95
TiO$_2$|Cobyrinate[‡]	--	0.21	0.98	1

[‡]Cobyrinate = dicyanocobyrinic acid heptamethyl ester, the Co(II/I) reduction potential for TiO$_2$|Cobyrinate was not directly observed as the presence of cyanide causes Co(II) to rapidly disproportionate, however it is expected to be similar to TiO$_2$|Cobalamin.[11,19]

It was found that the relative excited state electron transfer yield was wavelength-dependent, 532 and 683 nm excitation were within error of unity, but the yield for 436 nm excitation was only ~0.2. The reason for this is uncertain. The difference cannot be attributed to photo decomposition as there was no change in the visible absorbance spectrum after laser irradiation of any wavelength used. The visible absorption spectrum of cob(I)alamin has been attributed to corrin-localized $\pi\rightarrow\pi^*$ excitations.[20,21] However, recent computational and spectroscopic studies point toward considerable metal-to-ligand charge transfer (MLCT) character in the lower energy transitions.[22–26] The calculations of Brunold and coworkers indicate that the absorption at 436 nm consists of only 20% MLCT character, compared to 70% and >99% at 532 and 683 nm.[24] Thus, competitive absorption from $\pi\rightarrow\pi^*$ transitions may account for the significantly lower electron transfer yield measured after 436 nm excitation. Although the computed excited state energy of the $\pi\rightarrow\pi^*$ transition is thermodynamically capable of electron transfer to the acceptor states of TiO$_2$,[25] the MLCT is likely to be more directed, with a large change in the dipole moment. We note that the $\pi\rightarrow\pi^*$ excited states of other open-shell macrocycles, such as hemes, do not undergo excited state electron transfer reactions.[27]

The excited state reduction potential of cob(II/I*)alamin can be estimated from the measured Co(II/I) reduction potential and the free energy stored in the excited state.[28] The E^0(Co$^{II/I}$) was measured by spectroelectrochemistry, Table 1. As the free energy in the excited state is not known, the longest wavelength where MLCT transitions are expected to be significant by Brunold et al. (950 nm) was used as an estimate. This calculation yielded E^0(Co$^{II/I*}$) ≈ -1900 mV *vs.* NHE, a very potent reductant. The low energy absorption of the biomolecule raises the question of the consequences of possible *in vivo* excitation of cob(I)alamin to transiently form such a potent reductant.

Electron transfer from the excited state of an open-shell, first-row metal is uncommon, behaviour that is frequently explained by thermal deactivation of the excited state *via* low lying ligand field states. However, photo-induced electron transfer from d^8 transition metals has previously been observed suggesting that this deactivation pathway does not kinetically out-compete excited state electron transfer.[18] Although the photo-induced cleavage of the Co(III)-C bond in cobalamins has been known for some time, to our knowledge photo-induced outer sphere electron transfer has not been reported for any cob(I)alamin species.[29–37]

Conclusions

Vitamin B_{12} was anchored to TiO_2 and the cob(I)alamin state was produced electrochemically. Upon light excitation, excited state electron transfer yielded cob(II)alamin state with $k_{et} > 10^8$ s^{-1}. The excitation wavelength dependence suggested electron transfer from an MLCT excited state rather than a ligand localized $\pi \rightarrow \pi^*$ excited state. Despite the long fascination of biochemists and inorganic chemists alike, the essential cofactor cob(I)alamin has not previously been observed to undergo excited state electron transfer.

Notes and references

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