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Note

The reaction between methylcobalamin and cyanide revisited

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Abstract

The reaction between the vitamin B_{12} coenzyme methylcobalamin and cyanide has been re-investigated by UV–Vis and ^{1}H NMR spectroscopies under the same conditions. On the basis of the results and recent results of other studies on the reaction between other alkylcobalamins and cyanide, the changes in the ^{1}H NMR chemical shifts and linewidths in the aromatic region observed upon addition of cyanide to methylcobalamin can be attributed to the rapid formation of (β-CH_3)(α-CN)Cbl$^-$, rather than the formation of MeCbl·CN^- as proposed earlier (Inorg. Chem. 36 (1997) 4891). There is excellent agreement between the equilibrium constant for formation of (β-CH_3)(α-CN)Cbl$^-$ determined by the two methods (0.35 ± 0.03 M^{-1} and 0.31 ± 0.01 M^{-1} from the UV–Vis and ^{1}H NMR spectroscopic data, respectively (D_2O, pD 12.1, 25.0 °C)).
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1. Introduction

We recently investigated the reaction between methylcobalamin, MeCbl, and cyanide in aqueous and methanol solutions [1]. Significant changes in the ^{1}H NMR chemical shifts and linewidths of the B2 and B4 signals of MeCbl (and much smaller changes in the remaining signals which resonate in the aromatic region) were observed upon the addition of cyanide. It was proposed that MeCbl and cyanide can react to form an association complex of the type MeCbl·CN^-; that is, it was believed substitution by cyanide at either the α or β axial site of the cobalamin did not occur and the α and β ligands of the MeCbl remain unchanged.

However, more recent studies on the reaction between other alkylcobalamins and cyanide have led us to question our interpretation of the ^{1}H NMR spectroscopic data recorded for the reaction between MeCbl and cyanide in this paper [1,2]. Specifically, we have since shown that addition of 5′-deoxyadenosylcobalamin, AdoCbl [2a,3], (and also 5′-deoxyadenosylcobinamide [2b]) to cyanide results in the formation of an (β-adenosyl)(α-cyano)cobalamin intermediate ((β-Ado)(α-CN)Cbl$^-$) in both 92% DMF–8% D_2O and in aqueous solution. Cyanide can react with AdoCbl to form either a cyanocobalamin or (β-Ado)(α-CN)Cbl$^-$ intermediate, corresponding to substitution of the first cyanide at either the β or α site of AdoCbl, respectively. Comparison of the ^{1}H NMR and UV–Vis spectra of the intermediate with that of authentic cyanocobalamin showed that cyanide first substitutes at the α site to form (β-Ado)(α-CN)Cbl$^-$. In addition, the intermediate was light-sensitive, indicating that the β-5′-deoxyadenosyl ligand is still co-ordinated in the intermediate [2a]. Formation of (β-Ado)(α-CN)Cbl$^-$ from AdoCbl plus cyanide is rapid (i.e. fast exchange on the NMR time scale), so that only averaged signals are observed for {AdoCbl + (β-Ado)(α-CN)Cbl$^-$}. The significant absorbance of the γ band of the (β-Ado)(α-CN)Cbl$^-$ intermediate in the 580–620 region (AdoCbl and other β-alkylcobamides do not absorb significantly above 580 nm) has also been observed for a wide range of (β-alkyl)(α-cyano)cobamides [2].

We have since recorded UV–Vis spectra of equilibrated solutions of MeCbl and cyanide in aqueous

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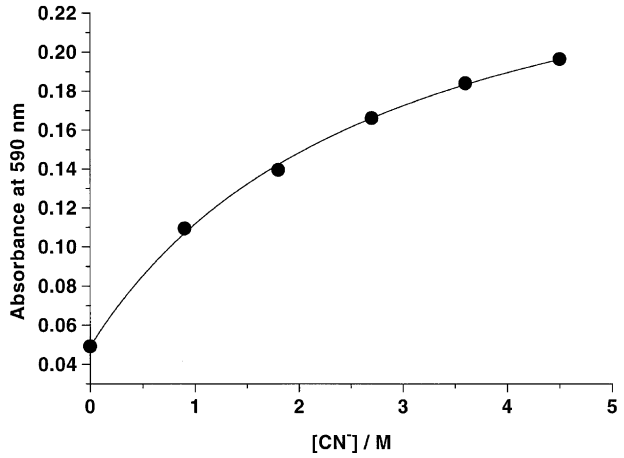

Fig. 1. Observed absorbance at 590 nm vs. cyanide concentration for the data given in Figure A in the Supplementary Material. The data has been fitted to Eq. (2) in the text (solid line), fixing $A_0 = 0.0492$ giving $K_{CN} = 0.35 \pm 0.03\ M^{-1}$ and $A_\infty = 0.289 \pm 0.009$.

solution up to cyanide concentrations of 6 M (Fig. 1, [2c]; $[CN^-] = 1$–6 M, pH 11.0, 25 °C). These UV–Vis spectra showed significant changes upon the addition of cyanide to MeCbl accompanied by isosbestic points at 325, 380, 455 and 553 nm, and a significant increase in the absorbance in the 580–650 nm region. We concluded on the basis of the large spectral changes and comparisons with UV–Vis spectral changes for other equilibrated solutions of alkylcobalamin plus cyanide that cyanide actually substitutes at the α-axial site of MeCbl; that is, (β-Me)(α-CN)Cbl$^-$ is formed. Association between MeCbl and CN^- to form MeCbl·CN^- is unlikely to give such large changes in the UV–Vis spectra.

These subsequent findings have led us to re-investigate the cause of the ^{1}H NMR chemical shift changes observed for the reaction between MeCbl and cyanide in our earlier paper [1], the results of which are presented herein. Based on the recent findings for other reactions involving alkylcobalamins and cyanide [2,3], it is more likely that the ^{1}H NMR spectral changes are instead associated with rapid formation of (β-Me)(α-CN)Cbl$^-$ rather than MeCbl·CN^-, and that MeCbl and (β-Me)(α-CN)Cbl$^-$ are in fast exchange at room temperature. To test this hypothesis, we set out to determine the value of the formation constant for (β-Me)(α-CN)Cbl$^-$ for equilibrated solutions of MeCbl and cyanide in D_2O by UV–Vis spectroscopy and to compare this value with the equilibrium constant obtained from ^{1}H NMR chemical shift data under the same conditions. If the two values are the same within experimental error, the chemical shift changes in MeCbl with changes in cyanide concentration can also be attributed to the rapid formation of (β-Me)(α-CN)Cbl$^-$, rather than formation of MeCbl·CN^- as proposed earlier [1].

Figure A in the Supplementary Material gives UV–Vis spectra for equilibrated solutions of MeCbl with 0–4.50 M NaCN in D_2O at pD 12.1 and 25 °C. Alkaline conditions were used to ensure all of the cyanide is present as CN^-, not HCN (pK_a(HCN) = 8.95 (25 °C, $I = 1.0$ M ($NaClO_4$)) [2b]. The spectral changes observed are essentially the same as those obtained in H_2O (Figure 1 in our earlier paper [2c]), for which the significant increase in the absorbance in the 580–650 nm region at high cyanide concentrations was attributed to the formation of (β-Me)(α-CN)Cbl$^-$. Since high cyanide concentrations are required for the formation of significant concentrations of the product, it was not feasible to work at constant ionic strength. The varying ionic strength did not appear to greatly affect the results (see below).

Fig. 1 gives a plot of absorbance at 590 nm versus cyanide concentration for the data shown in Figure A. Assuming that the spectral changes arise from MeCbl being converted to (β-Me)(α-CN)Cbl$^-$ as shown in Eq. (1)

$$CH_3Cbl + CN^- \overset{K_{CN},\ fast}{\rightleftharpoons} (\beta\text{-}CH_3)(\alpha\text{-}CN)Cbl^- \qquad (1)$$

it follows that

$$A_{obs} = A_0 + (A_\infty - A_0)K_{CN}[CN^-]/(1 + K_{CN}[CN^-]) \qquad (2)$$

where A_0 and A_∞ represent the absorbances of MeCbl and (β-Me)(α-CN)Cbl$^-$, respectively, and A_{obs} is the observed absorbance [4]. The best fit of the data to Eq. (2) gives $K_{CN} = 0.35 \pm 0.03\ M^{-1}$ at pD 12.1 and 25 °C. This value is the same within experimental error as the value of K_{CN} previously determined in H_2O ($K_{CN} = 0.38 \pm 0.03\ M^{-1}$ [2c]).

^{1}H NMR spectra were measured in D_2O under the same conditions as the experiments shown in Fig. 1, except that higher MeCbl concentrations were used to obtain an acceptable signal to noise ratio. We have previously shown that the aromatic region of the ^{1}H NMR spectrum is useful for characterising and quantitating cobalamin species in solution [5]. All signals in the aromatic region move downfield upon the addition of cyanide to MeCbl, with the greatest changes being observed for the B2 and B4 signals [6]. Similar changes in the ^{1}H NMR spectrum were observed upon the conversion of AdoCbl to (β-Ado)(α-CN)Cbl$^-$ in aqueous solution, with once again the B2 and B4 signals showing the greatest downfield shift [3]. This suggests that (β-Me)(α-CN)Cbl$^-$, not MeCbl·CN^-, is formed at high cyanide concentrations.

Plots of chemical shift versus cyanide concentration for the B2 and B4 signals of MeCbl (and (β-Me)(α-CN)Cbl$^-$) are given in Fig. 2. Assuming that MeCbl and (β-Me)(α-CN)Cbl$^-$ are in fast exchange (i.e., Eq. (1) applies), then

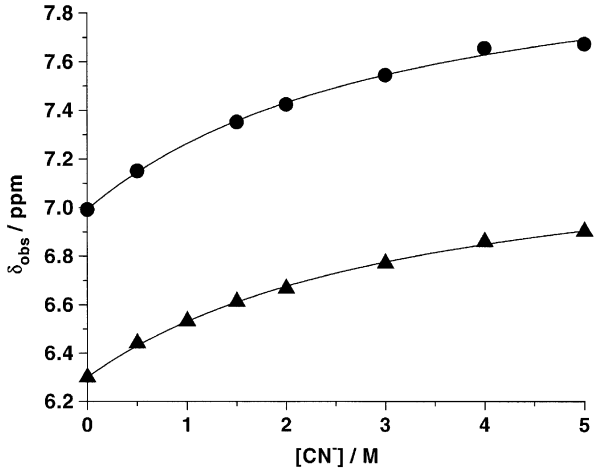

Fig. 2. Observed chemical shift vs. cyanide concentration for the B2 (●) and B4 (▲) signals of {MeCbl+(β-Me)(α-CN)Cbl$^-$}, pD 12.1±0.5, 25.0±0.5 °C. The data has been fitted to Eq. (3) in the text (solid line), fixing $\delta_{MeCbl} = 6.992$ for B2 and 6.300 for B4. This gave $K_{CN} = 0.31 \pm 0.04$ M^{-1} and $\delta_{(\beta\text{-Me})(\alpha\text{-CN})Cbl^-} = 8.14 \pm 0.07$ for the B2 data and $K_{CN} = 0.30 \pm 0.02$ M^{-1} and $\delta_{(\beta\text{-Me})(\alpha\text{-CN})Cbl^-} = 7.31 \pm 0.03$ for B4.

$$\delta_{obs} = \delta_{MeCbl} + (\delta_{(\beta\text{-Me})(\alpha\text{-CN})Cbl^-} - \delta_{MeCbl})K_{CN}[CN^-]/(1 + K_{CN}[CN^-]) \quad (3)$$

where δ_{obs} is the observed chemical shift, δ_{MeCbl} and $\delta_{(\beta\text{-Me})(\alpha\text{-CN})Cbl^-}$ represent the chemical shifts of MeCbl and (β-Me)(α-CN)Cbl$^-$, respectively, and K_{CN} is defined once again by Eq. (1). The data in Fig. 2 was fitted to Eq. (3), giving $K_{CN} = 0.31 \pm 0.04$ M^{-1} for the B2 data and 0.30 ± 0.02 M^{-1} for the B4 data. Both these values are in excellent agreement with the value of K_{CN} obtained from the UV–Vis spectral data ($K_{CN} = 0.35 \pm 0.03$ M^{-1}). This means that the changes observed by UV–Vis and ^{1}H NMR spectroscopies upon the addition of cyanide to a solution of MeCbl can be attributed to the formation of (β-Me)(α-CN)Cbl$^-$ in both cases. We, therefore, wish to replace our former interpretation that the changes observed by ^{1}H NMR spectroscopy for equilibrated solutions of MeCbl and cyanide are due to the formation of MeCbl·CN$^-$ [1]. They instead arise from the substitution of cyanide at the α-5,6-dimethylbenzimidazole site of MeCbl to form (β-Me)(α-CN)Cbl$^-$.

2. Experimental

Methylcobalamin (>97%) was purchased from Sigma, and sodium cyanide (≥98%) from May and Baker. All other reagents were AR grade. Distilled water was purified through a Milli-Q ultra-pure water system.

UV–Vis data was obtained using Cary 1E and Cary 5 spectrophotometers equipped with a thermostatted cell compartment (25.0±0.1 °C), operating with CARY WINUV BIO software, version 2.00. All spectra were measured in a 1 cm cuvette and samples were made up in D_2O. All preparation and handling of methylcobalamin solutions was carried out under diffuse or red-light-only conditions, and control experiments established that MeCbl is stable at pD 12.1 for the duration of the experiments.

Cyanide solutions were always freshly prepared. Some coloration of the cyanide solutions was observed at high cyanide concentrations, observable by UV–Vis spectroscopy for $\lambda < 400$ nm; however control experiments showed that <1% decomposition occurs within the time frame of both the UV–Vis and ^{1}H NMR experiments by titration of control solutions with silver iodide [7]. The spectra were analysed at 590 nm. pD 12.1 was used so as to avoid the addition of acid (HNO_3, $HClO_4$ or HCF_3SO_3) to the cyanide solutions since the addition of any of these acids to the cyanide solutions resulted in a substantial increase in the rate of decomposition of the cyanide solution. Thus, conditions were chosen so there was negligible cyanide decomposition.

^{1}H NMR spectra were recorded using an Inova 500 MHz NMR spectrometer equipped with a 5 mm thermostatted (25.0±0.5 °C) probe. Samples were prepared in D_2O with TSP as an internal reference and the watergate automatic solvent suppression sequence was employed. Samples were equilibrated in the probe for at least 10 min prior to collecting data.

pH measurements were made using a Mettler Toledo Wilmad 6030-M3 NMR pH electrode and an Orion model 710A pH/ISE meter. The meter and electrode were calibrated with BDH pH 6.98 and 10.00 standard buffers at room temperature. Solution pH was adjusted using conc. NaOH as necessary. The solution pD was calculated using the formula pD = pH + 0.40 [8].

Data were fitted to the appropriate equations using MICROCAL ORIGIN, version 3.5. Errors represent one standard deviation of the mean value.

3. Supplementary material

UV–Vis spectra for equilibrated solutions of MeCbl and NaCN in D_2O, pD = 12.1±0.3, 25.0±0.1 °C (Figure A) are available from N.E.B. on request.

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