Studies on the Mechanism of the Reaction between Coenzyme B<sub>12</sub> and Cyanide: Direct <sup>1</sup>H NMR Spectroscopic Evidence for a (β-5'-Deoxyadenosyl)(α-cyano)cobalamin Intermediate

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The reaction between coenzyme B<sub>12</sub> (5'-deoxyadenosylcobalamin, AdoCbl) and cyanide in aqueous solution. This study was carried out for a number of reasons: (1) The reaction between AdoCbl and cyanide results in quantitative heterolytic cleavage of alkylcobalamins in a key step. Factors which control the rate of Co–C bond cleavage of alkylcobalamins are poorly understood. Although an 1982 paper by Jencks and co-workers stands out in the prior alkylcobalamin plus cyanide literature, a paper we will return to later. (2) There is considerable discussion in the literature as to whether cyanide first substitutes at the (upper) axial site of AdoCbl (Figure 1).3–7 (3) Apart from... [text continues with references and discussion]
(1) Addition of the first cyanide was rate-determining under all conditions, so that all other subsequent steps to give the reaction products dicyanocobalamin ((CN)\textsubscript{2}Cbl\textsuperscript{-}), adenine, and the cyanohydrin 1-cyano-D-erythro-2,3-dihydroxy-4-pentenol are rapid. (2) The observed rate constant is acid-independent (unlike for acid-catalyzed heterolytic cleavage of AdoCbl\textsuperscript{12}). (3) Activation parameters (\(\Delta S^\ddagger\), \(\Delta V^\ddagger\)) typical for an associative rate-determining step were obtained. Since the reactants were converted directly to products without the build up of a monocyano intermediate, it was impossible to ascertain whether cyanide first substitutes at the \(\alpha\) or \(\beta\) axial site of AdoCbl. However, it was postulated on the basis of the activation parameters that cyanide was probably first substituting at the \(\beta\) site.\textsuperscript{1}

Further investigations on the AdoCbl plus cyanide reaction, but now in DMF (\(N,N\text{-dimethylformamide})/\text{D}_2\text{O}\) solvent mixtures, have resulted in the direct observation of the monocyano intermediate and are the subject of this note. The reasoning behind examining the reaction in a less protic solvent mixture is quite simple. Displacement of the adenosyl ligand by cyanide is accompanied by protonation of probably the ribosyl oxygen of the adenosyl ligand.\textsuperscript{13} This process is obviously rapid in aqueous solution (since the observed rate constant was found to be acid-independent\textsuperscript{1}); however in a less protic solvent mixture it was hoped that this process may now be slow enough to the extent that if cyanide first substitutes at the \(\beta\) site of AdoCbl, this step may now become acid-dependent. Alternatively, if cyanide first substitutes at the \(\alpha\) site of AdoCbl, then subsequent displacement of the Ado ligand by the second cyanide could be so slow that a (\(\beta\)-Ado)(\(\alpha\)-CN)Cbl\textsuperscript{-} intermediate could be observed. This latter case has, in fact, been found to occur.

### Results and Discussion

The reaction between AdoCbl and (TBA)CN (tetrabutylammonium cyanide) was examined in 92% DMF/\(8\% \text{D}_2\text{O}\) solutions using (TBA)\textsubscript{4}ClO\textsubscript{4} (tetrabutylammonium perchlorate) to maintain constant ionic strength (0.50 M).\textsuperscript{14} 1\textsuperscript{H} NMR spectroscopy proved to be a very informative way to follow the reaction.\textsuperscript{14} The 1\textsuperscript{H} NMR spectrum of AdoCbl in 92% DMF/\(8\% \text{D}_2\text{O}\) exhibits eight peaks at 8.26, 8.18, 7.33, 7.23, 6.56, 6.33 (d), 6.14, and 5.70 (d) ppm; three from the adenosyl ligand (A2, A8, A11 (d)), one from the corrin ring (C10), and four from the nucleotide (B2, B4, B7, R1 (d)).\textsuperscript{15} The chemical shifts are substantially different from those found for AdoCbl in \(\text{D}_2\text{O}\).\textsuperscript{15} Importantly, addition of a small amount of \(\text{D}_2\text{O}\) to the DMF not only made the reaction between AdoCbl and (TBA)CN convenient to follow (see later in discussion; the observed reaction is faster when more \(\text{D}_2\text{O}\) is present), but it greatly simplified interpretation of all 1\textsuperscript{H} NMR spectra by significantly reducing (\(\sim 95\%\)) the intensity of the amide side chain proton signals of the cobalamin, since proton–deuterium exchange between the amide protons and solvent \(\text{D}_2\text{O}\) occurs within minutes.


\textsuperscript{14} A recent paper of ours demonstrates just how useful the aromatic region of the 1\textsuperscript{H} NMR spectrum can be; see: Brasch, N. E.; Finke, R. G. J. Inorg. Biochem. 1999, 72, 215.

\textsuperscript{15} For the atom-numbering scheme and 1\textsuperscript{H} NMR shifts of AdoCbl in \(\text{D}_2\text{O}\) see: Summers, M. F.; Marzilli, L. G.; Bax, A. J. Am. Chem. Soc. 1986, 108, 4285.

\textsuperscript{16} The chemical shifts of the adenine signals were found to be concentration dependent: addition of 1.5 equiv of authentic adenine to the reaction product solution caused the 1\textsuperscript{H} NMR aromatic adenine signals to shift slightly downfield (8.06 and 7.83 ppm, respectively).

\textsuperscript{17} 1\textsuperscript{H} NMR spectra of the Co–C homolytic cleavage products 5'-deoxyadenosine and 8,5'-anhydroadenosine in 92% DMF/\(8\% \text{D}_2\text{O}\) have signals in the aromatic region around 8.41, 8.22, 6.04 (d), and 8.19 and 6.12 ppm, respectively (0.50 M (TBA)\textsubscript{4}ClO\textsubscript{4}, 25.0 °C). Addition of ca. 5.0 \(\times 10^{-2}\) M (TBA)CN to these solutions changed the chemical shifts by \(\pm 0.02\) ppm.
Evidence for a Cobalamin Intermediate

obtained by plotting ln(area of the (β-Ado)(α-CN)Cbl− signal at δ = 8.25 ppm) versus time for the experiment shown in Figure 2, giving \( k_{obs} = (8.5 \pm 0.4) \times 10^{-5} \text{ s}^{-1} \) (Figure A, Supporting Information). Additional HMOC and HMBC experiments made the assignment of the majority of the (β-Ado)(α-CN)Cbl− \(^1\)H NMR signals in the aromatic region possible (ppm): 8.34, B2, 8.25 and 8.18, A2 and A8; 7.49 and 7.40, B4 and B7; 6.42 (d), R1; 5.72 (d), A11; 5.65, C10.

The rate of conversion of (β-Ado)(α-CN)Cbl− to (CN)_2Cbl− in the presence of (TBA)CN could be considerably enhanced by exposing the solution to light, thus demonstrating that the intermediate contains a Co–C bond, prone to homolytic cleavage in the presence of light as is typical for adenosylcobalaminides. A \(^1\)H NMR spectrum of a freshly prepared solution of AdoCbl in 5.0 \( \times \) 10\(^{-2} \) M (TBA)CN which had been exposed to indirect sunlight for 1 h revealed that all cobalamin had been converted to (CN)_2Cbl− within this time. In the absence of light, the decomposition of the Ado ligand resulted in the Co–C heterolytic cleavage product adenine (and the cyanoheydrin).

Alternatively, the formation of the (β-Ado)(α-CN)Cbl− intermediate and its subsequent conversion to (CN)_2Cbl− can be followed by visible spectroscopy. Figure 3a gives visible spectra before and ca. 20 s after mixing an AdoCbl solution \( \lambda_{max} = 305, 337 \pm 2 \) (s), 375, and 519 nm) with a (TBA)CN solution in a tandem (split-cell) cuvette (5.0 \( \times \) 10\(^{-2} \) M (TBA)-CN, 92% DMF/8% D\(_2\)O, \( I = 0.50 \) M ((TBA)-ClO\(_2\)). A substantial change occurs before the first spectrum is obtained after mixing, indicating that a reaction has already occurred, namely, the rapid formation of the (β-Ado)(α-CN)Cbl− intermediate (\( \lambda_{max} = 329, 392, 468, \) and 578 nm). The \( \gamma^-\)band for the cobalamin moves to longer wavelengths (from 375 to 392 nm) as would be expected if the α-DMBI of AdoCbl was replaced by a cyanide ligand, confirming evidence that an α-cyano species is formed. By following the visible spectrum of the reaction mixture over time, one can monitor the conversion of (β-Ado)(α-CN)Cbl− to (CN)_2Cbl− (and adenine and cyanoheydrin) (see Figure 3b); \( \lambda_{max} \) for (CN)_2Cbl− = 314, 368, 420, 509 (s), 544, and 583 nm. The absorbance versus time data at a specific wavelength can be fitted to a first-order rate equation for the experiment shown in Figure 3, \( k_{obs} = (8.86 \pm 0.02) \times 10^{-5} \text{ s}^{-1} \) at \( \lambda = 625 \) nm (see Figure B, Supporting Information). Note that this value is in excellent agreement with the \(^1\)H NMR-determined value, \((8.5 \pm 0.4) \times 10^{-5} \text{ s}^{-1}\) (Figure A, Supporting Information).

\( k_{obs} = k_2[\text{CN}^-]/(1 + K[\text{CN}^-]) \) (1)

where the equilibrium constant \( K \) corresponds to rapid formation of the (β-Ado)(α-CN)Cbl− intermediate prior to rate-determining cleavage of the Co–C bond of the intermediate. Fitting the data in Figure 4 to eq 1 gives \( k_2 = (9.3 \pm 0.3) \times 10^{-5} \text{ s}^{-1} \) and \( K = (2.2 \pm 0.4) \times 10^2 \text{ M}^{-1} \).

The concentration dependence of the observed rate constant for the reaction between AdoCbl and cyanide was also examined by \(^1\)H NMR spectroscopy. Importantly, AdoCbl and (β-Ado)-(α-CN)Cbl− are in fast exchange by \(^1\)H NMR spectroscopy, since only 8, and not 16, signals were observed for a solution of ca. 1:4:1 (β-Ado)(α-CN)Cbl−:AdoCbl. The observed rate constant was found to be independent of CN− concentration.

Experiments examining the rate of the reaction between AdoCbl and cyanide at different cyanide concentrations were carried out in order to deconvolute the observed rate constants. The observed rate constant for the reaction between AdoCbl and cyanide obtained from visible spectroscopy measurements was found to be independent of cyanide concentration for \([\text{CN}^-] \geq 2.0 \times 10^{-2} \) M (Figure 4), but decreased with decreasing cyanide concentration for concentrations lower than this. Such curvature is typical of saturation kinetics, with a rate equation

\[ k_{obs} = k_2[\text{CN}^-]/(1 + K[\text{CN}^-]) \] (1)

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for CN\(^-\) = 2.00 \times 10^{-2} - 0.120 M \text{ M }^2 (Table A, Supporting Information), k\(_{obs}\) = (7.6 \pm 0.6) \times 10^{-5} s\(^-1\). This is not unexpected, since under these conditions (i.e., [CN\(^-\)] \geq 2.00 \times 10^{-2} M), only (\(\beta\)-Ado)(\(\alpha\)-CN)(\(\beta\)-Cbl)\(^-\) is present, and k\(_{obs}\) = k\(_2\) (eq 1). The value of k\(_2\) obtained by \(^1H\) NMR spectroscopy is in acceptable agreement (within 20\%) with the k\(_2\) value obtained by visible spectroscopy ((9.3 \pm 0.3) \times 10^{-5} s\(^-1\)), especially if one keeps in mind the slight differences when comparing results in deuterated and unlabeled solvents (i.e., DMF-d\(_7\) versus DMF).

Activation parameters for the cleavage of the Co–C bond of (\(\beta\)-Ado)(\(\alpha\)-CN)(\(\beta\)-Cbl)\(^-\) were also determined. The temperature dependence of the reaction between AdoCbl and CN\(^-\) in 92\% DMF/8\% D\(_2\)O (i.e., no HClO\(_4\)) is determined (Table A, Supporting Information). The k\(_{obs}\) was therefore examined under reaction conditions where the rate constant is independent of CN\(^-\); that is, k\(_{obs}\) = k\(_2\), \(
abla\DeltaH^\circ\) and \(\DeltaS^\circ\) were found to be 90.0 \pm 0.5 J mol\(^-1\) and 20.5 \pm 0.2 J mol\(^-1\) K\(^-1\), respectively.

A question which arises is does the present work, with its direct \(^1H\) NMR spectroscopic detection of an (\(\beta\)-Ado)(\(\alpha\)-CN)Cbl\(^-\) intermediate using 92\% DMF/8\% D\(_2\)O, have any relevance to the AdoCbl plus cyanide mechanism in aqueous solution?

(21) The \(^1H\) NMR spectrum of a freshly prepared solution of AdoCbl (5.1 \times 10^{-3} M) in ca. 9.3 \times 10^{-3} M (TBA)CN (92\% DMF-d\(_7\)=8\% D\(_2\)O, 25.0 °C, I = 0.50 M (TBA)-ClO\(_4\)) was recorded. Since K = 2.2 \times 10^{10} M\(^-1\), then under these conditions (\(\beta\)-Ado)(\(\alpha\)-CN)(\(\beta\)-Cbl)\(^-\) is ca. 1:4:1. The chemical shifts were found to be between those expected for AdoCbl and (\(\beta\)-Ado)(\(\alpha\)-CN)(\(\beta\)-Cbl)\(^-\); e.g., C10 5.84 (cf. C10(AdoCbl) = 6.14, C10(\(\beta\)-Ado)(\(\alpha\)-CN)(\(\beta\)-Cbl)\(^-\)) = 5.65).

(22) It was not possible to carry out \(^1H\) NMR experiments at [CN\(^-\)] < 2.00 \times 10^{-3} M, since at least 2 \times 10^{-3} M AdoCbl is required in solution to obtain an acceptable signal-to-noise ratio. CN\(^-\) concentrations were at least 10 times greater than this to ensure pseudo-first-order conditions.

Table 1. Dependence of the Observed Rate Constant for the Reaction between AdoCbl and Cyanide on the Percentage of D\(_2\)O in DMF/D\(_2\)O Solvent Mixtures (5.00 \times 10^{-2} M (TBA)CN, 25.0 °C)

<table>
<thead>
<tr>
<th>% D(_2)O</th>
<th>10^3kobs/s(^-1)</th>
<th>“isosbestic points”2H/nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0</td>
<td>0.33</td>
<td>349, 383, 475, 595</td>
</tr>
<tr>
<td>8.0</td>
<td>0.66</td>
<td>348, 384, 482, 598</td>
</tr>
<tr>
<td>15</td>
<td>1.5</td>
<td>347, 386, 486, 603</td>
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<tr>
<td>25</td>
<td>3.5</td>
<td>347, 386, 499, 607</td>
</tr>
<tr>
<td>50</td>
<td>5.9</td>
<td>347, 390, 528</td>
</tr>
<tr>
<td>100</td>
<td>5.9</td>
<td>347, 389, 531</td>
</tr>
</tbody>
</table>

In 92\% DMF/8\% D\(_2\)O cleavage of the Co–C bond of the \(\alpha\)-cyano intermediate is the rate-determining step (k\(_1\) = 9.3 \times 10^{-5} s\(^-1\)) (visible spectroscopy), 25.0 °C, I = 0.50 M ((TBA)-CN)); however in aqueous solution it was previously found that irreversible formation of the \(\alpha\)-cyano intermediate was rate-determining (k\(_1\) = 0.57 M\(^-1\) s\(^-1\), 25.0 °C, I = 1.0 M (NaClO\(_4\))). To test this, we examined the dependence of the observed rate constant on the percentage of D\(_2\)O in the DMF/D\(_2\)O solvent mixture. From Table 1 it can be seen that the “isosbestic points”22 change from four corresponding to the reaction of (\(\beta\)-CN)(\(\alpha\)-Ado)(\(\beta\)-Cbl) and cyanide to give (CN)(CN)(\(\alpha\)-Cbl)\(^-\) at low amounts of D\(_2\)O to three corresponding to the direct conversion of AdoCbl and CN\(^-\) to give (CN)(CN)(\(\alpha\)-Cbl)\(^-\) at high (>50\% D\(_2\)O) percentages of D\(_2\)O. Hence there is a changeover in the rate-determining step from rate-determining Co–C cleavage at the \(\beta\) site of the (\(\beta\)-Ado)(\(\alpha\)-CN)(\(\beta\)-Cbl)\(^-\) intermediate to rate-determining substitution of CN\(^-\) at the \(\alpha\) site of AdoCbl in solutions containing more D\(_2\)O than DMF.

From Table 1 it can also be seen that the rate of Co–C bond cleavage of the intermediate is more than two orders of magnitude smaller in 96\% DMF/4\% D\(_2\)O (3.3 \times 10^{-3} s\(^-1\)) compared with 100\% D\(_2\)O (>5.9 \times 10^{-3} s\(^-1\)); i.e., >10k\(_{obs}\), since this step is not rate controlling in 100\% D\(_2\)O. In addition, the rate constant for the reaction between AdoCbl and (TBA)CN (5.00 \times 10^{-2} M) in 92\% DMF/8\% H\(_2\)O was approximately 10\% larger (visible spectroscopy, four separate experiments gave 10^3k\(_{obs}\) = 7.69, 7.28, 7.66, and 7.65 s\(^-1\), respectively) than the rate constant obtained in 92\% DMF/8\% D\(_2\)O (visible spectroscopy, four separate experiments gave 10^3k\(_{obs}\) = 6.56, 6.53, 6.64, and 6.76 s\(^-1\), respectively). Both these results suggest that solvent is involved in the transition state preceding rate-determining cleavage of the Co–C bond.25 A mechanism involving solvent-assisted heterolytic Co–C cleavage (and labilization of the Co–C bond by the nucleophile trans to the alkyl group) for alkyldenalamines in alkaline solution was recently proposed by Finke and co-workers.26 An acid-independent path has also been found for Co–C heterolytic cleavage of alkyldenalamines under mildly acidic conditions (pH 2.7–5.5) by Halpern and co-workers, although the authors propose that the mechanism involves protonation of the ribosyl oxygen rather than the solvent supplying the proton (no (23) (a) From ref 1, k\(_1\) = k(1 + K\(_{CN}\)) = 0.57 M\(^-1\) s\(^-1\) using K\(_{CN}\) = 76.6 in H\(_2\)O, 25.0 °C.22 (b) Brown, K. L.; Hakimi, J. M.; Jacobsen, D. W. J. Am. Chem. Soc. 1984, 106, 7894.

(24) Upon close examination of the spectra it could be seen that the “isosbestic points” were not 100\% clean due to the decomposition of (TBA)CN (or an impurity in (TBA)CN) in DMF.29

(25) The proton inventory technique29 can give information on the number of solvent molecules involved in the transition state and involves determining k\(_{H2O}\)/k\(_{D2O}\) for a wide range of H\(_2\)O (or D\(_2\)O):dipolar aprotic solvent ratios. The reaction between AdoCbl and CN\(^-\) is not ideal for such a study, however, since Co–C cleavage is only rate-determining for H\(_2\)O (or D\(_2\)O):DMF < 50\%. (b) Espenson, J. H. In Chemical Kinetics and Reaction Mechanisms, 2nd ed.; McGraw-Hill: New York, 1995; p 219.

experiments were done to investigate this further).\(^{(27)}\) Interestingly, an acid-independent path was not found for the heterolytic cleavage of AdoCbl in mildly acidic solutions (pH 2–5).\(^{(28)}\)

The hereby modified\(^1\) proposed mechanism for the reaction between AdoCbl and cyanide (in the absence of light) is given in Scheme 1. In 92% DMF/8% D\(_2\)O rapid formation of an \(\alpha\)-cyano intermediate \((K_{CN})\) via a base-off AdoCbl \((1/K_{Co})\) is followed by rate-determining heterolytic Co–C cleavage \((k_2)\); thus the equilibrium constant \(K\) obtained by visible spectroscopy experiments, \(= K_{CN}/K_{Co}\).\(^{(29)}\) Addition of the second cyanide occurs after rate-determining Co–C cleavage and the \(\beta\)-riboyl oxygen of AdoCbl is not protonated prior to Co–C cleavage, since \(k_2\) was found to be independent of cyanide concentration and [H\(^+\)] concentration in the pH region studied, respectively. Rate-determining cleavage of the Co–C bond is proposed to be solvent-assisted, on the basis that \(k_2\) decreases significantly as the dipolar aprotic component of the solvent mixture is increased, in addition to being larger (ca. 10%) when D\(_2\)O is replaced by H\(_2\)O.

The formation of \((\beta\text{-alkyl})(\alpha\text{-cyano})\)cobalamins from the reaction of alkylcobalamins with cyanide in aqueous solution has been reported in the literature;\(^2\),\(^{26}\) however no values of \(K_{CN}/K_{Co}\) have been reported in other solvents. In their detailed mechanistic study of the reaction between cyanide and \(\beta\text{-oxocarbonyl})\)cobalamins \(((\text{methoxycarbonyl}methyl)\)cobalamin and (carboxymethyl)cobalamin), Jencks and co-workers found that fast, reversible formation of a \((\beta\text{-oxocarbonyl})(\alpha\text{-CN})\)cobalamin intermediate occurs prior to rate-determining heterolytic Co–C bond cleavage.\(^2\) In addition, Jencks et al. demonstrated that stereospecific protonation occurs in experiments using labeled, chiral cobalamin reactants; thus protonation of the \(\beta\text{-oxocarbonyl}\) leaving groups occurs after the rate-determining step but prior to separation of the \(\beta\) group from the cobalamin.

It was found that only eight averaged \(1\)H NMR signals were observed in the aromatic region for a ca. 1:4:1 mixture of \((\beta\text{-Ado})(\alpha\text{-CN})\)Cbl\(^-\)/AdoCbl; that is, the two species are in fast exchange. This means that the observed rate constant, \(k_{obs(1)}\), for the formation of the intermediate from AdoCbl and cyanide must be \(>100\) s\(^{-1}\). The observed rate constant \(k_{obs(1)}\) for the same reaction in 100% D\(_2\)O is \(5.9 \times 10^{-4}\) s\(^{-1}\) ([TBA]CN = 5.00 \(\times 10^{-3}\) M; see Table 1); therefore, in 92% DMF/8% D\(_2\)O the rate of cyanation is enhanced by over five orders of magnitude compared to that in D\(_2\)O. For biomolecular reactions involving small anions, rate enhancements of many orders of magnitude can occur when changing from a protic to a diprotic apolar solvent due to the increased activity of the anion (in this case, cyanide) as a result of its greatly reduced hydrogen-bonding interactions with the solvent.\(^{31}\) Some of the rate enhancement may also be attributable to more AdoCbl existing in its base-off form in 92% DMF/8% D\(_2\)O.

There has been much discussion in the literature over factors which are important for Co–C heterolytic bond cleavage in alkylcobalamins, as opposed to those which lead to efficient Co–C homolytic bond cleavage. Our study confirms that the \(\alpha\)-bonded ligand trans to the alkyl group plays a key role in Co–C heterolytic bond cleavage. In addition, whereas protonation of the ribosyl oxygen of AdoCbl is crucial prior to Co–C heterolytic cleavage in acidic solution,\(^12\) in more alkaline conditions in the presence of cyanide the rate of Co–C heterolytic cleavage of the \((\beta\text{-Ado})(\alpha\text{-CN})\)Cbl\(^-\) intermediate is

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\(^{(29)}\) \(K_{Co}\) would need to be determined in 92% DMF/8% D\(_2\)O to allow the calculation of \(K_{CN}\). \((K_{Co} = 76.6\) in H\(_2\)O, 25.0 °C.\(^{23}\)\)


acid-independent and the solvent supplies the proton. The reason why trans-bound cyanide and imidazolate promote Co–C heterolysis would appear to be due to their strong σ electron donor abilities, leading to stabilization of the Co–C heterolytic cleavage transition state. Kräutler has also proposed that a Co─Cα─Cβ─O trans antiperiplanar conformation in the alkyl group is extremely favorable for Co–C heterolysis. To summarize, the reaction between AdoCbl and cyanide has been examined by 1H and visible spectroscopies in 92% DMF/8% D2O solutions and shown to involve a (β-Ado)(α-CN)Cbl− intermediate. Under these conditions Co–C bond cleavage of the α-cyano intermediate is the rate-determining step rather than addition of the first cyanide as found in aqueous solution. The kinetics of the reaction have been examined as a function of cyanide concentration by both 1H and visible spectroscopies, and the latter shows saturation kinetics demonstrating that the formation of the α-cyano intermediate from AdoCbl and cyanide is a reversible process. Additional experiments have also been carried out which show that Co–C cleavage is solvent-assisted. There is a changeover in the rate-determining step when more D2O is used in the DMF/D2O solvent mixture, until for D2O ≥ 50% addition of the first cyanide is rate-determining. These results, then, unify the mechanism of Co–C heterolytic cleavage of alkylcobalamins by cyanide (and presumably other nucleophiles such as imidazolates8), implicating both the trans effect and solvent-assisted Co–C heterolysis as important factors involved in the Co–C heterolytic cleavage process of alkylcobalamins in alkaline solution.

Experimental Section

(TBA)CN (96%) and 5′-deoxyadenosine (97%) were purchased from Aldrich, and (TBA)ClO4 (≥99%) and adenine (≥99%) from Fluka. [Caution: (TBA)CN is highly toxic.] AdoCbl (98%) and CNCbl (99%) were obtained from Sigma. 8,5′-Anhydroadenosine was prepared by a modified published procedure,8 in which 5 mol equiv of KCN was added and the pH adjusted to ca. 8.5, instead of the reported addition of both KCN and HCN. It was found unnecessary to wash the column with 1% HCN.

Visible spectra were measured using a Cary 1E spectrophotometer equipped with a thermostated cell compartment (25.0 ± 0.1 °C). 1H NMR spectra were obtained on a Varian Inova 500 MHz spectrometer equipped with a 5 mm thermostated (25.0 ± 0.2 °C) probe. TSP was added as an internal reference.

(TBA)CN was stored under nitrogen and handled in a glovebag under positive nitrogen pressure. All solutions prepared for 1H NMR measurements were made up in DMF-d7. Unlabeled DMF was used for visible spectroscopy experiments. D2O was used for both 1H NMR and visible spectroscopy measurements unless explicitly stated otherwise. All solutions were filtered through a Millipore filter (0.45 μm).

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Supporting Information Available: A plot of ln(peak area, δ = 8.25 ppm) versus time corresponding to the experiment shown in Figure 2 (Figure A), plot of absorbance at 625 nm versus time for the experiment shown in Figure 3 (Figure B), and table giving observed rate constants for the reaction between AdoCbl and cyanide determined by 1H NMR spectroscopy (Table A). This material is available free of charge via the Internet at http://pubs.acs.org.

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(34) Bernhauer, K.; Müller, O. Biochem. Z. 1961, 335, 44.