Inorg. Chem. **1983**, *22*, 2635–2637

Contribution from the Department of Chemistry,
Kent State University, Kent, Ohio 44242

Electron Transfer. 64. Reduction of Nitrate by Vitamin B_{12s} (Cob(I)alamin)[1]

P. N. BALASUBRAMANIAN and E. S. GOULD*

Received January 26, 1983

Vitamin B_{12s} (cob(I)alamin), the Co(I) derivative of vitamin B_{12}, reduces nitrate rapidly and cleanly to NH_4^+ at pH 1.5–2.5. The overall rate for this net transfer of eight electrons is determined by the initial step. The observed rate law, rate = $k[Co^I][NO_3^-][H^+]$ ($k = 2.1 \times 10^4$ M^{-2} s^{-1}; 25 °C; $\mu = 0.11$), is consistent with initiation either by reaction of the nonprotonated form of B_{12s} with molecular HNO_3 or by hydride transfer to NO_3^- from the protonated form of B_{12s}. Observed variation of the Co(II) (B_{12r}) spectrum with acidity leads to a calculated pK_A of 1.96 for the latter system.

Interest in the chemistry of vitamin B_{12s}, the cobalt(I) derivative of vitamin B_{12}, has taken several diverse directions. Early work was spectrochemical in nature,[2] and a subsequent body of evidence has accumulated, pointing to the intervention of this species in enzymatic transformations.[3] The synthetic utility of this complex has been exploited,[4] and its behavior as a nucleophile has been examined in detail.[5] Somewhat more recently, attention has been turned to aspects of its electrochemistry[6] and to its participation in electron-transfer reactions.[7]

The present paper deals with the reduction of nitrate to ammonia by cob(I)alamin, a net 8e transaction for which indirect electrochemical evidence has been reported.[6b] This study is not without implications pertaining to nitrogen metabolism in biosystems.

Experimental Section

Materials. Sodium nitrate (Baker Analyzed grade) and hydroxocobalamin hydrochloride (Sigma) were used as received. Lithium perchlorate, for use in kinetic experiments, was prepared as described.[8] All reactions were carried out under N_2 in distilled water that had been previously boiled and purged with O_2-free nitrogen for at least 4 h.

Cob(I)alamin was generated in solution by a modification of the procedure of Heckman and Espenson,[9] which was used to prepare the cobalt(II) complex, vitamin B_{12r}. Solutions of the cobalt(III) complex, hydroxocobalamin hydrochloride, were acidified and then reduced by stirring with amalgamated zinc in 0.1 M $HClO_4$ at room temperature. In our hands, the peaks at 312 and 470 nm, which are characteristic of Co(II),[10] developed quickly and then underwent rapid decay while the peaks at 387, 560, and 680 nm, which are characteristic of Co(I), grew steadily and then reached a constant height less than 5 min after the reduction was initiated. Observed spectral features of B_{12r} and B_{12s} at low pH are summarized in Table I.

Stoichiometric Studies. The stoichiometry of the reaction between cob(I)alamin and nitrate in 0.010 M $HClO_4$, with the Co^I complex in excess, was determined by adding a measured deficiency of NO_3^- to the Co^I solution, waiting 20 min for completion of the reaction, and then measuring the decrease in absorbance at 387 nm. This decrease was then compared to that observed when Co^I was treated with excess nitrate. Representative results appear in Table II. Very nearly 8 mol of Co^I reacts with each mole of nitrate under the conditions indicated. Solutions resulting from treatment of cob(I)alamin with a tenfold excess of nitrate gave positive Nessler tests[11] for ammonia.

Kinetic Studies. Rates were estimated from measurements of decreases in absorbance at 387 nm using a Cary 14 recording spectrophotometer. The difficulty in transferring dilute solutions of cob(I)alamin led us to adopt the procedure described by Loar,[12] in which the reductant was generated directly in the 1.00-cm spectrophotometric cell by use of a small weighed quantity of zinc amalgam and a 7-mm Teflon-coated magnetic stirrer. For kinetic runs, the spectrophotometer beam was positioned above the level of the amalgam so that disturbances in the solid did not result in optical interference. Stirring was discontinued just before addition of the oxidant. Kinetic runs generally required 3 min, or less, during which time reduction of Co^{II} produced in the primary reaction to Co^I by the unstirred amalgam was negligible, as demonstrated by very nearly constant infinity values in the kinetic curves obtained.

Assignment of an $[H^+]$ value for each kinetic run was complicated by gradual losses of acidity resulting from the Co^I-catalyzed reaction of zinc with H^+.[5] Acidities were therefore estimated either by titration of an aliquot of the reaction mixture just before addition of nitrate or by use of a predetermined logarithmic $[H^+]$-decay curve applicable to the system at hand. Estimates based on the two methods were in agreement. For most runs 0.06 M $LiClO_4$ was added as a supporting electrolyte, but in a few instances where the concentration of this salt was raised to 0.20 M, no significant acceleration was observed, thus ruling out an appreciable Co^I–ClO_4^- reaction (in agreement with the findings of Itabashi and co-workers[6b]). Reactions were first order each in Co^I and NO_3^- but were generally carried out under pseudo-first-order conditions with the ratio $NO_3^-:Co^I > 2$ (resulting in the loss of less than $^1/_{16}$th of the added nitrate). Conversions were followed for at least 5 half-lives. Rate constants from successive half-life values within a run agreed to within 5%, and average values did not differ significantly from those obtained from slopes of logarithmic plots of absorbance differences against reaction times. Specific rates from replicate runs checked to better than 9%. Examination of kinetic profiles gave no indication of the intervention of an intermediate formed or destroyed on a time scale comparable to that for the measured disappearance of Co(I). In particular, no sharp drop in absorbance occurred immediately after addition of NO_3^-, thus ruling out the rapid formation of a partially reduced nitrogen species. Temperatures were kept at 25.0 ± 0.2 °C during the entire series of experiments.

Cob(I)alamin did not react with excess acetone or acetonitrile under our conditions. On the other hand, reactions with *p*-nitrophenol, azobenzene, a series of $(NH_3)_5Co^{III}$ complexes, and the ordinarily sluggish oxidant $Co(en)_3^{3+}$ were each too rapid to follow by using ordinary mixing techniques. The isomeric dicyanobenzenes appeared to consume 4 equiv of Co^I at measurable rates; the latter reactions are being pursued.

Results and Discussion

Earlier examinations of the B_{12r}–B_{12s} system[6a,13] point to the partition, in the pH range considered here, of the Co(II) complex into a unipositive protonated form, II_{BH}^+, and a

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(1) Support of this work by the National Science Foundation (Grant No. 8022881) is gratefully acknowledged.

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(11) Swift, E. H. "A System of Chemical Analysis"; Prentice-Hall: Englewood Cliffs, NJ, 1940; p 403.

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(13) Lexa, D.; Saveant, J.-M. *J. Chem. Soc., Chem. Commun.* **1975**, 872.

0020-1669/83/1322-2635$01.50/0

Table I. Electronic Spectra of the Cobalt(II) and Cobalt(I) Derivatives of Vitamin B_{12} in Aqueous $HClO_4$

	λ_{max}, nm (ϵ)^a	
	obsd	lit.^{b}
Co^{II} (B_{12r}) 0.040 M H^+ ^{c}	470 (1.3), 403 sh (0.72), 312 (3.1)	473 (1.1),^{d} 405, 312
0.0005 M H^+	473 (0.74), 402 (0.63), 310 (2.8)	
Co^I (B_{12s}) 0.040 M H^+ ^{e}	680 (0.20), 560 (0.35), 460 (0.43), 387 (3.0)	680, 554, 460, 385 (3.1)^d
Co^I-Co^{II} isosbestic points, 0.040 M H^+	540, 415, 355, 305	

^{a} ϵ values in M^{-1} cm^{-1} $\times 10^{-4}$; sh = shoulder. ^{b} Reference 10. ^{c} Solution was prepared by oxidation of B_{12s} with excess nitrate. ^{d} Reference 7c. ^{e} Prepared by reduction of B_{12a} with zinc amalgam (see Experimental Section).

Table II. Stoichiometry of the Reaction of Cob(I)alamin with Nitrate^a

$10^5[Co^I]$, M	$10^6[NO_3^-]$, M	$10^5\Delta[Co^I]$, M^b	$\Delta[Co^I]$/ $\Delta[NO_3^-]$
8.48	6.70	5.14	7.67
6.36	7.20	5.62	7.81
6.48	6.00	4.76	7.93
6.36	6.00	4.95	8.25
6.36	3.60	3.08	8.54

^{a} Cob(I)alamin was generated by reduction of hydroxocobalamin hydrochloride with zinc amalgam. Reductions were carried out in 0.010 M $HClO_4$ (see Experimental Section). ^{b} Determined spectrophotometrically at 387 nm.

Table III. Representative Kinetic Data for the Reduction of Nitrate by Vitamin B_{12s} (Cob(I)alamin)^a

$10^5[NO_3^-]$, M	$10^3[H^+]$,^{b} M	μ	10^2k_{obsd},^{c} s^{-1}
6.8	12.6	0.11	2.8
6.2	12.6		2.7
14.0	12.6		4.2
30.8	12.6		8.3
14.9	6.3	0.11	1.76
14.0	18.9		6.3
14.5	25.1		7.6
14.7	31.5		10.1
14.9	12.6	0.21	3.6
14.9	12.6	0.41	3.3

^{a} Reactions were carried out at 25.0 °C; the supporting electrolyte was $LiClO_4$. $[Co^I]_0 = 6.3 \times 10^{-5}$ M throughout. ^{b} $[H^+]$ values corrected for loss of acidity during generation of Co(I) (see Experimental Section). ^{c} $k_{obsd} = (-d[Co^I]/dt)[Co^I]^{-1}$.

neutral deprotonated form, II_B, the latter featuring internal coordination of side-chain benzimidazole at an axial site on Co^{II}. (A second deprotonated "base-off" form comprises only 1.6% of Co^{II} at that protonation level and does not appear to play a significant part in this study.) In the same range, Co^I exists predominantly as a neutral monoprotonated species, I_{BH} (pK_A = 4.7), which can undergo further protonation to a unipositive ion, $I_{BH_2}{}^+$ ($pK_A \sim 1$), at very high acidities.

The observed approach to 8:1 stoichiometry in our systems (Table II), in conjunction with the identification of ammonia as the reaction product, tells us that the principal net reaction is

$$8Co^I + NO_3^- + 10H^+ \rightarrow 8Co^{II} + NH_4^+ + 3H_2O \quad (1)$$

Although this net transfer of eight electrons must occur in a series of steps, we find no hint of the intercession of an intermediate reacting at a rate commensurate with that of the primary reaction. The straightforward exponential decay curves observed with NO_3^- in excess indicate that the overall rate is being determined by the first step in the sequence, the initial attack on NO_3^-, i.e. that nitrate is reduced more slowly by Co(I) than is any of its reduction products.[14]

Representative kinetic data for the Co(I)-NO_3^- reaction appear in Table III. The reduction is seen to be first order in both NO_3^- and H^+, conforming to rate law 2. The activated

$$\text{rate} = k[Co^I][NO_3^-][H^+]$$
$$k = 2.1 \times 10^4\ M^{-2}\ s^{-1};\ 25\ °C;\ \mu = 0.11 \quad (2)$$

complex for the rate-determining step thus features one unit each of Co^I (the uncharged species I_{BH}), NO_3^-, and H^+. The observed negative kinetic salt effect (see the final two entries in Table III) is in accord with an activation process in which ions of opposite charge are brought together. Our experiments give no information as to the progress of the reaction after the rate-determining step.

Two reaction sequences consistent with eq 2 come to mind. The first is initiated by reaction of the uncharged complex of Co^I with the small concentration of un-ionized nitric acid in solution;[15] the second involves reaction of the acidic form of cob(I)alamin, $I_{BH_2}{}^+$, with NO_3^-.[16,17] Of the several reported mechanistic studies pertaining to the reactions of nitrate with reducing metal centers,[18] only one, dealing with the Cr(II)-NO_3^- system,[18a] mentions a first-order H^+ dependence. This is taken to imply a path in which Cr^{2+} reacts directly with HNO_3, and the recorded rates, in conjunction with the reported K_A (15 M) for HNO_3,[17] lead to a calculated bimolecular specific rate of $2.2 \times 10^2\ M^{-1}\ s^{-1}$ for the Cr^{2+}-HNO_3 reaction (1 M ClO_4^-). Analogous treatment of the present system leads to a bimolecular rate constant of $3.2 \times 10^5\ M^{-1}\ s^{-1}$ for the suggested Co^I-HNO_3 reaction (0.11 M ClO_4^-). If it is further assumed that both Cr^{2+} and Co^I are acting as outer-sphere 1e reductants and that both conform to the Marcus[19] model, only a portion ($10^{1.7}$) of the $10^{3.2}$-fold difference in specific rates may be attributed the more negative potential of the Co^I complex.[20,21] The remaining advantage may reasonably be

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(14) This situation is analogous to that observed for the reduction of nitrate by Fe(II) in basic media. See, for example: Brown, L. I.; Drury, J. S. *J. Chem. Phys.* **1967**, *46*, 2833.

(15) For a mechanism featuring the reaction of un-ionized HNO_3, the observed retardation with increasing ionic strength may be interpreted as a secondary salt effect, i.e. one resulting from a decrease in the (small) equilibrium concentration of this acid.

(16) The apparent absence of kinetic saturation with respect to H^+ in the concentration range examined does not differentiate between these possibilities since this range falls well below both the acidity constant of cob(I)alamin (0.1)[13] and that of HNO_3 (15).[17]

(17) Davis, W., Jr.; de Bruin, H. J. *J. Inorg. Nucl. Chem.* **1964**, *26*, 1069. These authors present detailed evidence supporting the indicated value in preference to the older spectroscopically determined acidity constant of 22 M (Hood, G. C.; Redlich, O.; Reilly, C. A. *J. Chem. Phys.* **1954**, *22*, 2067). For further consideration of this point, see: Petkovic, D. M. *J. Chem. Soc., Dalton Trans.* **1982**, 2425.

(18) See, for example: (a) Ogino, H.; Tsukahara, K.; Tanaka, N. *Bull. Chem. Soc. Jpn.* **1974**, *47*, 308 (reduction by Cr^{2+}). (b) Guyman, E. D.; Spence, J. T. *J. Phys. Chem.* **1966**, *70*, 1964 (Mo^V). (c) Haight, G. P., Jr.; Katz, A. *Acta Chem. Scand.* **1962**, *16*, 659 (Sn^{II}). (d) Polyakova, V. M.; Chernyaskaya, E. I. *Tr. Inst. Khim., Akad. Nauk SSSR, Ural. Fil.* **1970**, *94*; *Chem. Abstr.* **1971**, *75*, 155312*a* (Ti^{III}). (e) Vicente-Perez, A.; Cabrera-Martin, A.; Gonzalez-Martin, C. *Quim. Anal.* **1976**, *30*, 149; *Chem. Abstr.* **1977**, *86*, 132723*y* (Ni^I). (f) Moyer, B. A.; Meyer, T. J. *J. Am. Chem. Soc.* **1979**, *101*, 1326 (Ru^{II}).

(19) Marcus, R. A. *J. Chem. Phys.* **1956**, *24*, 966, 979. It is assumed here that an increase in ΔG^* resulting from an increase in the standard potential of the reductant is half the increase in $\Delta G°$. (See, for example: Hush, N. S. *Trans. Faraday Soc.* **1961**, *57*, 557.)

(20) Lexa[6a] records the formal potential of the Co^I-Co^{II} system under consideration as -0.851 V vs. SCE or -0.609 V vs. NHE. The $E°$ value for $Cr^{2+/3+}$ is taken as -0.41 V.[21]

taken to reflect a greater self-exchange rate associated with the corrin-derived reductant.

The alternate sequence, featuring reaction of protonated cob(I)alamin with NO_3^-, would not, on the basis of experience with more usual redox systems, be seriously considered, for it is recognized that protonation of an oxidant almost invariably converts it to a more effective acceptor,[22] whereas protonation of a reductant renders it less strongly reducing. However, the perceived hydride-like character of Co^{I}–H species[23] makes this mechanism a reasonable one. Thus, we cannot ignore the possibility that the reaction is initiated by a hydride transfer (formally a 2e transaction) to nitrate (eq 3) followed immediately by a Co^{I}–Co^{III} comproportionation (eq 4). The specific

$$Co^{I}\text{–}H + NO_3^- \rightarrow NO_2^- + Co^{III} + OH^- \quad (3)$$

$$Co^{III} + Co^{I} \rightarrow 2Co^{II} \quad (4)$$

rate for the latter transfer has been shown[7b] to be much greater than that for the reaction being considered here. Sequence (3)–(4) is closely similar to that proposed for the reduction of N_2O by B_{12s},[24] and it may be that reaction of Co(I) with aqueous acid to give H_2 proceeds, at least in part, in an analogous fashion. Additional points in favor of such a path are that the overall conversion to NH_3 is accomplished in four, rather than eight, transactions[25] and that it bypasses zerovalent nitrogen, which, in its diatomic form, should be reduced with difficulty.[26]

Note finally that the spectrum of the Co^{II} product exhibits significant variation with acidity (see Table I), reflecting the equilibrium between the protonated and nonprotonated species, II_{BH}^+ and II_B. This variation is most marked within the pH range 1.5–2.5, with a limiting spectrum observed near $[H^+] = 4 \times 10^{-4}$ M. A plot of $(A_{obsd} - A_{lim})$ vs. the quotient $(A_{obsd} - A_{lim})/[H^+]$ (where the A values refer to absorbances at 470 nm) is closely linear and yields, as its slope,[27] the acidity constant 0.0109 M ($pK_A = 1.96$). The value, which refers to 0.11 M ClO_4^-, may be compared to the recorded[6a] pK_A of 2.9, the latter measured in 1.6 M aqueous *p*-toluenesulfonate.[28,29]

Acknowledgment. The authors are grateful to Dr. James Espenson for initiating interest in this work and for valuable discussions.

Registry No. NO_3^-, 14797-55-8; HNO_3, 7697-37-2; vitamin B_{12s}, 18534-66-2; vitamin B_{12r}, 14463-33-3.

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(21) Grube, G.; Breitinger, G. *Z. Elektrochem.* **1927**, *33*, 112. See also: Latimer, W. M. "Oxidation Potentials", 2nd ed.; Prentice-Hall: Englewood Cliffs, NJ, 1952; p 248.

(22) Exceptional cases involve inner-sphere oxidants in which the lead-in sites can be blocked off by protonation. See, for example: Loar, M. K.; Thomas, J. C.; Reed, J. W.; Gould, E. S. *Inorg. Chem.* **1977**, *16*, 2877.

(23) See, for example: Dodd, D.; Johnson, M. D. *J. Organomet. Chem.* **1973**, *52*, 1.

(24) (a) Banks, R. G. S.; Henderson, R. J.; Pratt, J. M. *J. Chem. Soc. A* **1968**, 2886. (b) A similar route has been suggested for the reduction of nitrate by Cr(II); see: Swaddle, T. W. *J. Am. Chem. Soc.* **1967**, *89*, 4338.

(25) Implicit in this argument is the assumption that if a 2e change is the preferred path for the initial step, it is also the preferred path for subsequent steps.

(26) A reviewer has pointed out that this must be so, since the entire series of reactions is carried out under N_2.

(27) See, for example: (a) Benesi, H. A.; Hildebrand, J. H. *J. Am. Chem. Soc.* **1949**, *71*, 2703. (b) Ramette, R. W. *J. Chem. Educ.* **1967**, *44*, 647.

(28) Preliminary experiments indicate that HNO_2 undergoes a 6e reduction by cob(I)alamin at a specific rate near 10^3 M^{-1} s^{-1} (25 °C; $\mu = 0.10$; $[H^+] = 0.016$ M), i.e. that the reaction is considerably faster than the reduction of NO_3^- under comparable conditions.

(29) Two additional points raised by a reviewer merit response. First, there is a suggestion that eq 3 might be rewritten in terms of Co^{I}–H and undissociated HNO_3 to avoid the release of OH^- in acidic solution. It appears to us that, unless these protonated species were formed in a highly unusual manner, such a step would be reflected in a reaction rate proportional to $[H^+]^2$ under our conditions. It was further noted that the species HNO or NOH is a much more likely N(I) intermediate than N_2O, for although the latter is reduced by B_{12s},[24a] this reduction is slow compared to the reaction at hand. On this point we agree.

Contribution from the Institute of Physical Chemistry,
Chalmers University of Technology, S-412 96 Gothenburg, Sweden

Optical Activity of Bis(thiocarbamide)bis(amino acid)platinum(II) Complexes

OLEG P. SLYUDKIN[†] and BENGT J. F. NORDÉN*

Received May 26, 1982

Unidentate amino acid platinum(II) complexes of the type *trans*-$[Pt(ZH)_2(Thio)_2]Cl_2$, where ZH = L-alanine (I), L-valine (II), L-tyrosine (III), and L-hydroxyproline (IV, V) and Thio = thiocarbamide, have been synthesized. Their electronic absorption and circular dichroism (CD) spectra have been studied in aqueous and in dimethylformamide (DMF) solution. A reversible change of CD with pH is observed for the alanine, valine, and tyrosine complexes in DMF. Similar CD spectra of DMF solutions of the complexes + 2 equiv of OH^- probably represent the contribution from the resulting asymmetric (deprotonated) nitrogen. With hydroxyproline (in contrast to the other amino acids) the complexes isolated from acid and alkaline solutions have quite different CD spectra, which is interpreted in terms of opposite absolute configurations of the asymmetrically coordinated nitrogen of the hydroxyproline ligand: Pt(S_N,S_C-hydroxyproline) (acidic medium), Pt(R_N,S_C-hydroxyproline) (alkaline medium) (S_C refers to C-2 of 4-hydroxyproline). This hypothesis is supported by chemical correlations and CD spectra of other proline complexes.

Introduction

Circular dichroism (CD) in the region of the d–d transitions of conformationally or vicinally chiral, planar metal complexes has been the subject of intense study (see e.g. Ref 1). Although the optical activity of planar complexes with chelated amino acids[2–4] has been studied in detail, very little has been done on unidentate amino acid complexes.[4] Such studies are of interest for understanding the mechanism of induction of chirality in d–d transitions. This kind of information could

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[†] On leave from the Inorganic Chemistry Department, Novosibirsk State University, 630090 Novosibirsk, USSR.

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(4) Volstein, L. M.; Slyudkin, O. P. *Zh. Neorg. Khim.* **1980**, *25*, 231; **1981**, *26*, 991; *Russ. J. Inorg. Chem.* (*Engl. Transl.*) **1980**, *25*, 125; **1981**, *26*, 496.

0020-1669/83/1322-2637$01.50/0