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# Interaction of Hexacyanoferrate(II) with Cyanocobalamin. Kinetics and Reaction Mechanisms

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*In acidic media, the kinetically stable cyanocobalamin was observed to undergo rapid substitution in accordance to the rate law*

$$k_{obs} = a + bT_{Fe}$$

*with a and b correlated to the backward and forward processes, respectively. The mechanism of substitution is discussed. The equilibrium constant for the acid dependent path is 9.3 × $10^3$ and for the independent path is 4.75 × $10^2$.*

## Introduction

Various aspects of the biochemical action of cobalt corrinoids have been reported [1, 2]. Surprisingly, not a great deal of work has been published on the kinetics of ligand substitution in cobalamins. Few works have investigated the displacement of the water molecule in aquacobalamin (3–5) by the ligands azide, cyanate, imidazole, thiocyanate, sulfite, thiosulphate, iodide and bromide. These reactions are rapid in contrast to the fact that octahedral Co(III) complexes are inert. Thusius [5], in contrast to Randall and Alberty [3, 4], has concluded that the dissociation of water in the *trans* position in aqua-cobalamin is the rate determining step followed by rapid addition of the entering ligand.

It has been also known that the ligand trans to a σ-bonded alkyl substituent undergoes substitution at rates considerably higher than the same ligand in similar compounds lacking M–C bond [6, and references therein]. The kinetic expressions which have been exposed to examination (in such cases) take one of the following forms:

*Scheme I.* A limiting $S_N1$ mechanism

$$R(Co)H_2O \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} R(Co) + H_2O$$

$$R(Co) + L \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} R(Co)L$$

$$k_{obs} = \frac{(k_1k_2/k_{-1})(L) + k_{-2}}{1 + (k_2/k_{-1})(L)} \quad (L \gg R(Co)H_2O) \quad (1)$$

*Scheme II.* Outer-sphere complexation mechanism

$$R(Co)H_2O\ L \underset{}{\overset{K_{iP}}{\rightleftharpoons}} R(Co)H_2O\cdots L$$

$$R(Co)H_2O\cdot\cdot L \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} R(Co)L + H_2O$$

$$k_{obs} = \frac{(k_3 + k_{-3})\,K_{iP}(L) + k_{-3}}{1 + K_{iP}(L)} \quad (L \gg R(Co)H_2O) \quad (2)$$

In fact, reported experimental kinetic data for the equilibrium

$$R(Co)H_2O + L = R(Co)\,L + H_2O$$

is of the form

$$k_{obs} = k_f(L) + k_b \quad (3)$$

Comparing eq. (3) with eqs. (1) and (2) indicates that either $(k_2/k_{-1})\,L \ll 1$ in scheme 1 or $K_{iP}(L) \ll 1$ in scheme II.

In the present work we report the results of the kinetics of interaction of hexacyanoferrate(II) with cyanocobalamin in acidic solution.

## Experimental

*Reagents*

Analytical grade $K_4Fe(CN)_6 \cdot 3H_2O$ and Vitamin $B_{12}$ (cyanocobalamin) were used without further purification. A stock solution of hexacyanoferrate(II) (0.10 *F*) was freshly prepared in all kinetic runs. Stock solution of 1.4 × $10^{-3}$ *F* cyanocobalamin was prepared and kept in a dark container.

Kinetic runs were carried out using a Durrum/Gibson Stopped-Flow apparatus thermostatically con-

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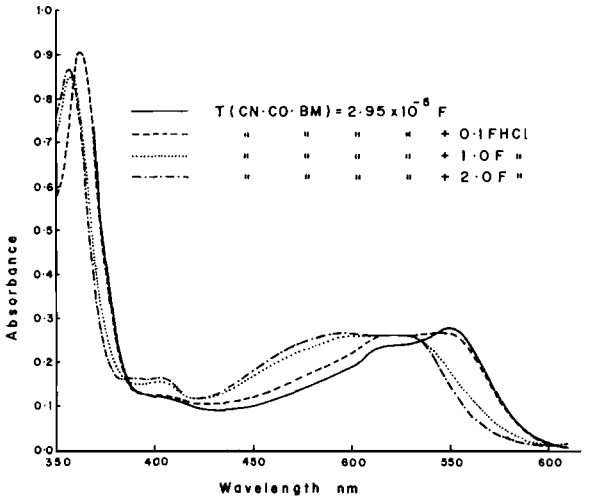



Figure 1. Spectra of cyanocobalamin at different acid concentrations.

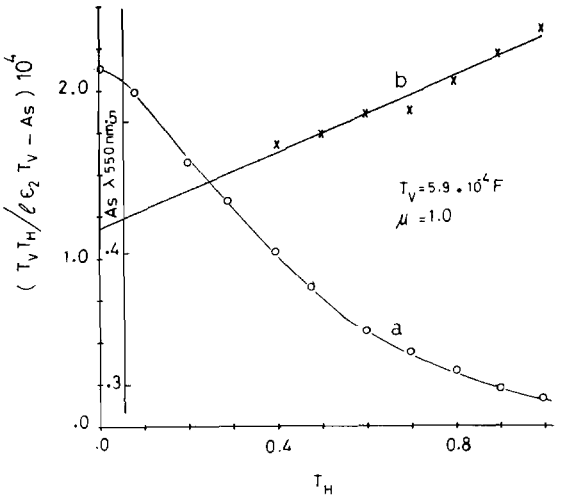



Figure 2. (a) The absorbance of cyanocobalamin as function of $T_H$ at λ 550 nm and $\mu$ = 1.0 *F* at 25 °C. (b) The plot of $(T_V T_H / l\epsilon_2 T_V - As_i)$ as function of $T_H$.

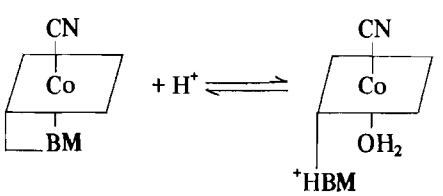
trolled at 25 °C. Optical path-length was 2.0 cm. The wavelength used was 550 nm.

The spectrum of vitamin $B_{12}$ in presence and absence of hexacyanoferrate(II) at different total acid concentrations were recorded on Sp 8000 PYE–UNICAM double beam spectrophotometers. The concentration range of cyanocobalamine was (2.95–5.90) $10^{-5}$ *F* and that of hexacyanoferrates was (0.4–2.0) $10^{-3}$ *F*. The total hydrochloric acid concentration was in the range 0.1–1 *F*. Ionic strength was kept at constant value of 1.0 *F* using the appropriate mixtures of HCl and KCl solutions.

## Results and Discussion

A change in color (pink to orange pink) has been observed when cyanocobalamin was acidified with HCl solution. The spectra of cyanocobalamin solutions at different hydrogen ion concentrations are shown in Fig. 1. The bands are slightly shifted to shorter wavelengths as hydrogen ion concentrations increases. When hexacyanoferrate(II) solution was added to the acidified solution of cyanocobalamin a rapid change to the original color has been observed.

Earlier work had indicated that cyanocobalamin is protonated in acidic media [1]. The nitrogen atom of α-5,6-dimethylbenzimidazole nucleotide group *trans* to the cyano ligand is readily protonated in acidic medium with its position being occupied




(4)

by a water molecule or chloride ion. The protonation equilibrium constant was reported to be ~0.8 [1]. Other protonation constants are of very small magnitude. This value has been checked further in this work. The spectra of cyanocobalamin were taken at different hydrochloric acid concentrations. The absorbance at 550 nm was taken as function of the total acid concentrations ($T_H$) shown in Fig. 2(a). The protonation constant may be calculated from the following equation

$$\frac{(H)_i T_V}{l\epsilon_2 T_V - As_i} = \frac{1}{K_V \Delta\epsilon} + \frac{(H)}{\Delta\epsilon} \qquad (5)$$

where $T_V$ is the total concentration of cyanocobalamin; $As_i$ the absorbance at a given wavelength; $\Delta\epsilon = \epsilon_2 - \epsilon_1$ where $\epsilon_1$ and $\epsilon_2$ are the molar absorptivities of the protonated and nonprotonated species of cyanocobalamin and $K_V$ the protonation constant.

The molar absorptivity $\epsilon_2$ was taken from the absorbance value of cyanocobalamin only in 1 *M* KCl, and (H) was assumed to be equal to the total acid concentration. By plotting the left hand-side term *versus* $T_H$ a linear plot has been obtained with intercept and slope equal to (1.13 ± 0.07) $10^{-4}$ and (1.18 ± 0.10) $10^{-4}$, respectively, Fig. 2(b). The calculated value of $K_V$ amounts to 1.04 ± 0.11, which is not different significantly from the reported value of ≅0.80.

On the other hand, numerous protonated species of hexacyanoferrate(II) coexist in solution. In fact, 5 species may exist as the following equilibria indicate

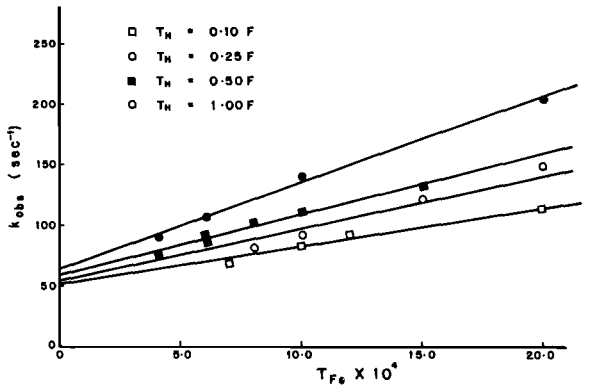



Figure 3. The dependence of observed rate constant on both acid concentration and hexacyanoferrate(II).

a) $Fe(CN)_6^{4-} + H^+ \rightleftharpoons HFe(CN)_6^{3-}$;

$$K_A = \frac{[HFe(CN)_6^{3-}]}{[H^+]\,[Fe(CN)_6^{4-}]}$$

b) $HFe(CN)_6^{3-} + H^+ \rightleftharpoons H_2Fe(CN)_6^{2-}$;

$$K_B = \frac{[H_2Fe(CN)_6^{2-}]}{[HFe(CN)_6^{3-}]\,[H^+]}$$

(6)

c) $H_2Fe(CN)_6^{2-} + H^+ \rightleftharpoons H_3Fe(CN)_6^-$;

$$K_C = \frac{[H_3Fe(CN)_6^-]}{[H_2Fe(CN)_6^{2-}]\,[H^+]}$$

d) $H_3Fe(CN)_6^- + H^+ \rightleftharpoons H_4Fe(CN)_6^o$;

$$K_D = \frac{[H_4Fe(CN)_6^o]}{[H_3Fe(CN)_6^-]\,[H^+]}$$

Few reports studied the above equilibria [7], however only $K_A$ and $K_B$ have been determined. The values obtained for $K_A$ and $K_B$ are $5.2 \times 10^2$ and $0.83 \times 10^2$, respectively. The third and forth equilibria are expected to have constants of very low values (<0.1).

The kinetic study was carried out at the wavelength 550 nm in the acid concentration range of 0.1–1.0 *F*. Figure 3 shows the dependence of observed rate constant, $k_{obs}$, on both $T_H$ and total concentration of hexacyanoferrate, $T_{Fe}$. At a given acid ($T_H$) concentration, $k_{obs}$ is linearly dependent on $T_{Fe}$ (Fig. 3) *i.e.*,

$$k_{obs} = a + bT_{Fe} \qquad (7)$$

The values of a and b are listed in Table I. Interaction of hexacyanoferrate(II) species with that of cyanocobalamin may follow scheme III.

At a given hydrogen ion concentration, the rate equation describing the mechanism in scheme(III) can be expressed as follows:

TABLE I. The Values of the Constants a and b at Different Acid Concentrations.

| $T_H$, *F* | a, sec$^{-1}$ | b X 10$^{-4}$, sec$^{-1}$ $M^{-1}$ | R |
|---|---|---|---|
| 0.10 | 51.2 ± 6.5 | 3.24 ± 0.49 | 0.978 |
| 0.25 | 53.4 ± 6.6 | 4.38 ± 0.48 | 0.988 |
| 0.50 | 56.4 ± 4.6 | 5.43 ± 0.49 | 0.988 |
| 1.00 | 63.2 ± 5.2 | 7.24 ± 0.44 | 0.996 |

$$-\frac{d[(I)+(II)+(III)+(IV)+(V)]}{dt} = -Z\frac{d(V)}{dt} =$$

$$= \frac{T_{Fe}(V)}{X}\left[\frac{(H^+)U}{K_V} + W\right] - (V)(H^+)M + N \qquad (8)$$

where

$$X = (H^+)^4 + (H^+)^3K_A + (H^+)^2K_AK_B + (H^+)K_AK_BK_C + K_AK_BK_CK_D,$$

$$U = k_1(H^+)^4 + k_2(H^+)^3K_A + k_3(H^+)^2K_AK_B + k_4(H^+)K_AK_BK_C + K_AK_BK_CK_D,$$

$$W = k_6(H^+)^4 + k_7(H^+)^3K_A + k_8(H^+)^2K_AK_B + k_9(H^+)K_AK_BK_C + K_{10}K_AK_BK_CK_D,$$

$$M = k_{-1}\frac{(H^+)^4}{Q_I} + k_{-2}\frac{(H^+)^3}{Q_{II}} + k_{-3}\frac{(H^+)^2}{Q_{III}} + k_{-4}\frac{(H^+)}{Q_{IV}} + k_{-5}$$

$$N = k_{-6}\frac{(H^+)^4}{Q_I} + k_{-7}\frac{(H^+)^3}{Q_{II}} + k_{-8}\frac{(H^+)^2}{Q_{III}} + k_{-9}\frac{(H^+)}{Q_{IV}} + k_{-10}$$

$$Z = \frac{(H^+)^4}{Q_I} + \frac{(H^+)^3}{Q_{II}} + \frac{(H^+)^2}{Q_{III}} + \frac{(H^+)}{Q_{IV}} + I$$

where $k_i$'s are the forward rate constant, $k_{-i}$'s are the backward rate constants, I, II, III, IV, V are the product species, and $Q_I$, $Q_{II}$, $Q_{III}$, $Q_{IV}$ are the overall deprotonation constants of I, II, III, IV species. The vertical protolytic reactions of the above scheme are assumed to be at equilibrium since they are fast in comparison with the complex formation reactions. Since the total acid concentration is not altered during the reaction progress, the integrated expression of eq. 9 can be obtained

$$\ln\frac{(V)_{eq}}{(V)_{eq} - (V)_t} = k_{obs}t \qquad (9)$$

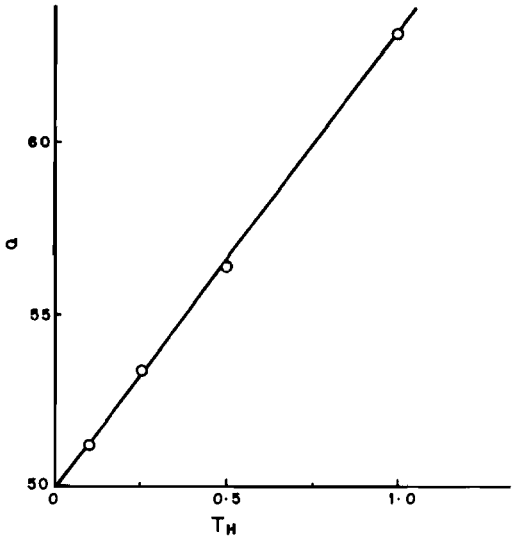



Figure 4. The dependence of a on hydrogen ion concentration.

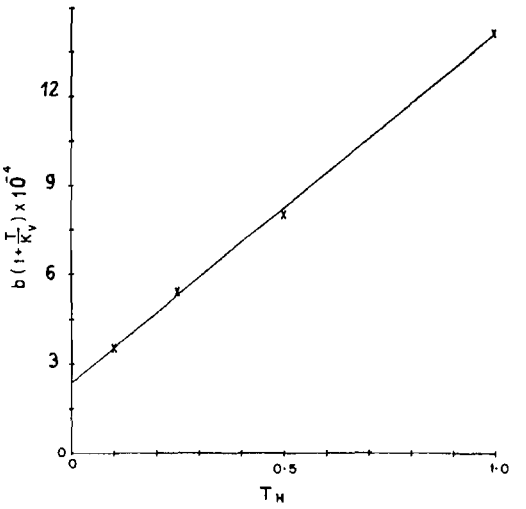



Figure 5. The dependence of $b(1 + T_H/K_V)$ on hydrogen ion concentration.

where $(V)_{eq}$ and $(V)_t$ are the concentrations of the complex at equilibrium and at time t and

$$k_{obs} = \left[\left(\frac{(H^+)U}{XK_V} + \frac{W}{X}\right)\Big/\left(1 + \frac{(H^+)}{K_V}\right)\right]T_{Fe} + \frac{(H^+)M + N}{Z}$$

The observed rate constant, $k_{obs}$, was obtained experimentally from the slope of the plot of $\ln(As_{eq}/As_{eq} - As_t)$ *vs.* t when $T_{Fe} \gg T_V$. The derived rate constant can be correlated with that obtained experimentally (eq. (7)), *i.e.*

$$a = \frac{(H^+)M + N}{Z} \tag{10}$$

$$b = \left[\frac{(H^+)U}{XK_V} + \frac{W}{X}\right]\Big/\frac{1 + (H^+)}{K_V} \tag{11}$$

The validity of eq. 8 and the linear dependence of $k_{obs}$ on $T_{Fe}$ indicate that the rate of complex reaction is first order with respect to cyanocobalamin and hexacyanoferrate concentration. Moreover the linear dependence (Fig. 4) of 'a' on hydrogen ion concentration indicates that Z is practically equal to one. From the slope and intercept of the straight line, the M and N values were obtained (M = 13.2 ± 0.2 and N = 49.9 ± 0.1).

The foregoing conclusion indicates further that the terms

$$k_{-1}\frac{(H^+)^4}{Q_I}, k_{-2}\frac{(H^+)^3}{Q_{II}}, k_{-3}\frac{(H^+)^2}{Q_{III}} \text{ and } k_{-4}\frac{(H^+)}{Q_{IV}}$$

may be ignored with respect to $k_{-5}$ and the terms

$$k_{-6}\frac{(H^+)^4}{Q_I}, k_{-7}\frac{(H^+)^3}{Q_{II}}, k_{-8}\frac{(H^+)^2}{Q_{III}}, k_{-9}\frac{(H^+)}{Q_{IV}}$$

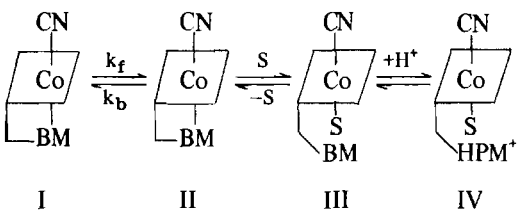
may also be ignored with respect to $k_{-10}$. On the other hand, the dependence of b on hydrogen ion concentration looks complicated as eqn. 10 indicates. However, little rearrangement may show the dependence

$$b\left[1 + \frac{(H^+)}{K_V}\right] = \frac{U}{XK_V}(H^+) + \frac{W}{X} \tag{12}$$

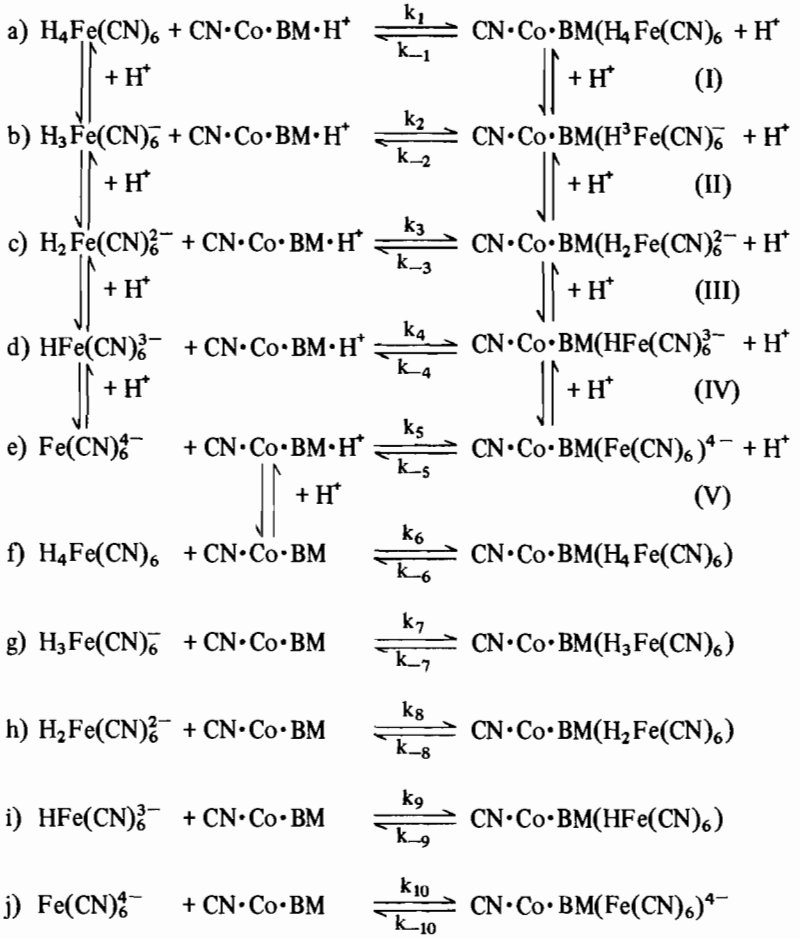
The plot of the left-side term of eqn. 12 *versus* $(H^+)$ showed a linear dependence, Fig. 5, with intercept (= W/X = (2.37 ± 0.15) × $10^4$) and slope (= U/$XK_V$ = (11.80 ± 0.26) $10^4$). This conclusion indicated that the terms $U/XK_V$ and W/X are independent of hydrogen ion concentration. In other words, $U/XK_V$ should be reduced to $k_5/K_V$ from which $k_5$ can be calculated by knowing $K_V$, *i.e.*

$$k_5 = (1.23 \pm 0.13)10^5 \text{ and } \frac{W}{X} = k_{10}.$$

From the foregoing results, one can reach the conclusion that reactions e and j are quite significant in the above reaction mechanism. In scheme (III), we have not accounted for the rapid equilibrium which may exist between pentacoordinate and hexacoordinate species of cyanocobalamin or base-off and base-on forms [8]. If these forms are taken in consideration two routes may be distinguished:



*Scheme (III)*




One route in which species (I) and (II) react with hexacyanoferrate(II) species. Another route involves the reaction of species (III) and (IV) with the hexacyanoferrate(II) species. Since the observed rate was only detected in acidic solution one may exclude the former route and substitution by the hexacyanoferrate(II) species should involve species (III) and (IV). In such case, it is not simple to differentiate between a limiting $S_N1$ mechanism or outer-sphere complexation mechanism.

If our kinetic conclusions are correct the equilibrium constants of reactions e and j in scheme (III) can be calculated. The values so obtained are $K_e = k_5/k_{-5} = (9.30 \pm 0.99)\ 10^3$ and $K_j = k_{10}/k_{-10} = (4.75 \pm 0.30)\ 10^2$.

The values of $K_e$ and $K_j$ are expected to be of equal magnitude since $K_v = 1.04$, but $K_e/K_j = 20$. This difference can be seen as resulting from the formation of an outer-sphere complex in reaction (e) in scheme III. The reaction path j in scheme III is probably dissociative.

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