Kinetics of Cobalamin Substitution Reactions

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Abstract: Rate constants for the formation and dissociation of a number of cobalamins, CBM–L (L = SCN^-, SO_3^{2-}, $S_2O_3^{2-}$, NCO^-, N_3^-, I^-, and Br^-), have been determined using fast-reaction techniques. The formation rate constants are nearly the same order of magnitude (170–2300 M^{-1} sec^{-1}), whereas the dissociation rate constants range between 590 and $\lesssim 10^{-5}$ sec^{-1} at 25°. The results are consistent with a transition state in which both entering and leaving groups are at most loosely bound to the cobalt atom. It was found that the large rate differences between aquocobalamin and the kinetically inert model cobalamin complexes, iodo- and nitroaquobis(dimethylglyoximato)cobalt(III), arise mostly from differences in activation entropies.

Although a great deal of rate data exist on the substitution reactions of hexaaquo and other simple coordination compounds,[2,3] detailed kinetic studies on macrocyclic complexes such as metalloporphyrins and corrinoids have been initiated only recently. For a number of reasons the cobalamins (cobalt(III) corrinoids) are particularly interesting as a subject for kinetic investigation: (1) cobalamins are water soluble and exist as monomers in solution; (2) chelation with the benzimidazole side chain prevents formation of 2:1 complexes; (3) three-dimensional structures of a number of corrinoids have been determined;[4,5] (4) stability constants for the association of a variety of ligands with aquocobalamin have been determined and the optical properties of the complexes characterized;[6] (5) substitution reactions of Co(III) complexes have been studied extensively and many kinetic data are available on these systems.

In the present study formation and dissociation rate constants for a series of cobalamins have been determined using stopped-flow and temperature-jump techniques. In most cases activation parameters are also reported. Two papers by Randall and Alberty have appeared on ligand binding to aquocobalamin.[7,8] Three of the reactions studied by these authors—the binding of N_3^-, OCN^-, and SCN^-—are also reported on here, but at several temperatures and, in the case of SCN^- and N_3^-, over a larger range of ligand concentration. Although the N_3^- and OCN^- results are in satisfactory agreement with those of Randall and Alberty, the SCN^- reaction exhibited more complex kinetics than found in the earlier work.[7,9] The latter system will be discussed in more detail at a later date.

Experimental Section

Materials. Reagent grade sodium thiocyanate was recrystallized twice from water and the concentration of stock solutions was determined by titration with $AgNO_3$. Solutions of sodium bisulfite were prepared by the dropwise addition of perchloric acid to a solution containing an equivalent amount of analytical grade sodium sulfite. The sulfite concentration was determined by titration with iodine. Stock solutions of sodium thiosulfate (A.R. grade) were also analyzed iodometrically. All other chemicals were either analytical or reagent grade, solutions being prepared from a known weight (or volume) of reagent.

Crystalline hydroxocobalamin chloride (Glaxo) was the source of aquocobalamin. The absorption at 350 mμ was used to determine the concentration of stock solutions.[6] Decomposing the aquo complex to the dicyano species and reading the absorption at 365 mμ gave the same concentration. Solutions were prepared from freshly boiled, doubly distilled water and stored in the dark at 5°.

Kinetics. Most anation reactions were followed in the visible region by means of a Durrum–Gibson stopped-flow apparatus having a cell path length of 2 cm and a dead time of about 3 msec. A ligand solution of the desired pH and at an ionic strength of 0.5 M (adjusted with sodium perchlorate or potassium nitrate) was mixed with an aquocobalamin solution of the same pH and ionic strength. In all cases the ligand concentration after mixing was at least 100 times greater than that of the aquocobalamin, the latter usually being $2–5 \times 10^{-5}$ M. The pH was adjusted either with acetate buffer or by titration with dilute NaOH or $HClO_4$. Total buffer concentration was never greater than 0.05 M and had no apparent effect on the reaction rates.

To ensure that $\lesssim 1\%$ of the cobalamin was in the form of the hydroxo complex, the pH was always kept below 6.0. In addition, a lower pH limit was placed on many of the reactions due to either protonation of the ligand or to decomposition of the ligand or complex.[6,8] Anation rates were constant between the following investigated pH values: SCN^-, 3.9–5.4; I^-, 4.2–5.7; $S_2O_3^{2-}$, 4.8–6.0; HSO_3^-, 3.7–5.8; Br^-, 3.1–5.8.

Iodide solutions were prepared fresh each day and stored under nitrogen. Without these precautions a two-step reaction was observed, presumably due to a side reaction involving I_3^-.

The aquation reactions were initiated by rapidly mixing a NaOH solution (usually 0.05 M) with an unbuffered or weakly buffered cobalamin solution of pH 4–5 which contained enough ligand to place $\gtrsim 80\%$ of the cobalt in the complexed form. The aquation of the azido and thiosulfato complexes were followed with a Cary 14 spectrophotometer.

First-order rate constants and reciprocal relaxation times were determined from plots of log $(I_t - I_\infty)$ *vs.* time, where I is the intensity of the transmitted light as measured in mV. $(I_t - I_\infty)$ is proportional to $(OD_t - OD_\infty)$ when $(I_t - I_\infty)/I_\infty < 0.1$. This condition was satisfied in the T-jump experiments by virtue of the small perturbation of the equilibrium state and was maintained in the stopped-flow runs by working at an appropriate wavelength. Plots were made from three or more different oscillograms and the time constants averaged. The average deviation from the mean was usually about 5%.

The temperature-jump apparatus was of single-beam design. Discharging a 5.0×10^{-7} F capacitor loaded to 9000 V effected a temperature rise of 12° in *ca.* 100 μsec. The size of the jump was determined by measuring the change in absorbance of a cresol red solution following the discharge. The cell path length was 7 mm and the volume was 2 ml. An apparatus with a tenfold higher resolution time ($C = 5 \times 10^{-8}$ F) was used to estimate the lower

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(2) M. Eigen and R. G. Wilkins, *Advan. Chem. Ser.*, No. 19, 25 (1965).

(3) F. Basolo and R. G. Pearson, "Mechanisms of Inorganic Reactions," 2nd ed, Wiley, New York, N. Y., 1968.

(4) D. C. Hodgkin, *Proc. Roy. Soc., Ser. A*, **288**, 294 (1965).

(5) J. D. Dunitz and E. F. Meyer, *ibid., A*, **288**, 361 (1965).

(6) J. M. Pratt and R. G. Thorp, *J. Chem. Soc. A*, 187 (1966).

(7) W. C. Randall and R. A. Alberty, *Biochemistry*, **5**, 3189 (1966).

(8) W. C. Randall and R. A. Alberty, *ibid.*, **6**, 1520 (1967).

(9) The SCN^- reaction exhibits three relaxation times,[10] whereas only one effect was detected in ref 7. This discrepancy can be attributed to the negligible amplitude of two of these effects at the ligand concentrations employed in the earlier study.

(10) D. Thusius, *Chem. Commun.*, 1183 (1968).

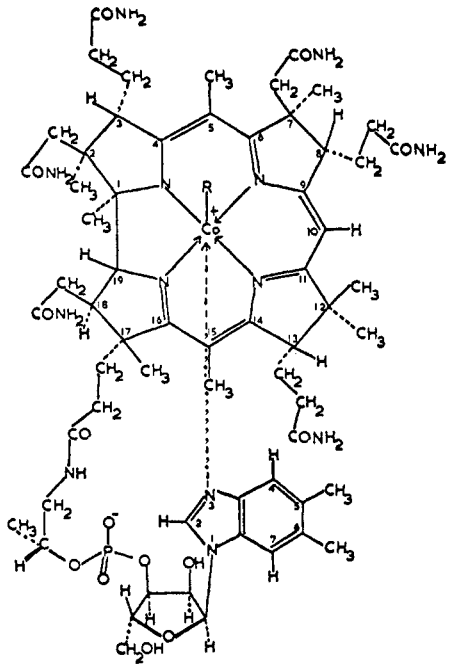

Figure 1. The structure of the cobalamin molecule. The ligand R bears a formal charge of 1−.

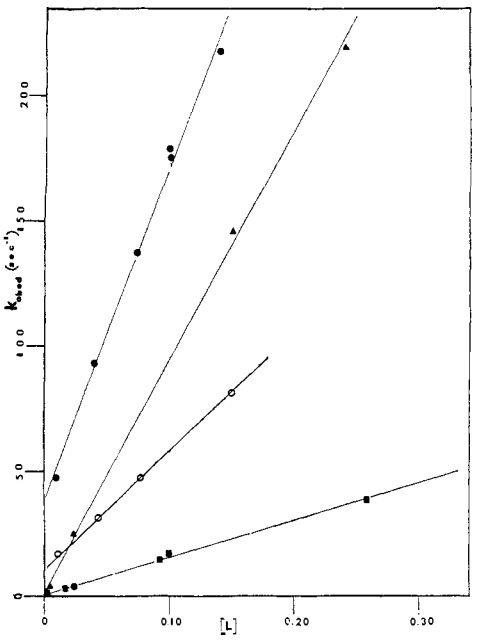

Figure 2. Ligand dependence of some aquocobalamin anation reactions: ●, I^- (25.5°); ○, I^- (12.7°); ▲, N_3^- (25.5°); ■, HSO_3^- (25.5°).

limit of the rate of absorption change at saturating ligand concentrations.

Difference spectra were obtained by thermostating the sample and reference compartments of the Cary spectrophotometer independently and scanning with a slidewire for the 0.0–0.1 absorbance range. The base line was determined by running water against water at the same ΔT.

Results

(a) **Anation and Aquation Kinetics.** With the exception of L = HSO_3^- (see below) the stoichiometry of the reactions under consideration is[6-8,11,12]

$$L + CBM\text{-}OH_2 \underset{k_d}{\overset{k_f}{\rightleftharpoons}} CBM\text{-}L + H_2O \quad (1)$$

Although the ambident nature of many of the ligands can result in a mixture of isomers, there is reason to believe that the complexes formed from SCN^-, $S_2O_3^{2-}$, and HSO_3^- are predominantly sulfur bound;[6,11] NCO^- is probably nitrogen bound.[6]

The rate equation for eq 1 is

$$-d(CBM\text{-}OH_2)/dt = k_f(L)(CBM\text{-}OH_2) - k_d(CBM\text{-}L) \quad (2)$$

If (L) ≫ (CBM-OH_2), it can be shown that

$$-\ln [(CBM\text{-}OH_2)_t - (CBM\text{-}OH_2)_\infty] = k_{app}(t) - \ln [(CBM\text{-}OH_2)_0 - (CBM\text{-}OH_2)_\infty] \quad (3)$$

$$k_{app} = k_f(L) + k_d \quad (4)$$

Formation rate constants at 25° were evaluated from plots of k_{app} *vs.* (L). In most cases the intercept was too small to yield an accurate value for the dissociation rate constant. Some of the results are presented in Figure 2.

In general, one-step reactions were observed. With thiocyanate, however, the overall reaction becomes biphasic when (SCN^-) is raised above 0.03 *M*, apparently due to an isomerization of the complex.[10]

The stoichiometry of the bisulfite reaction is very probably[11]

$$HSO_3^- + CBM\text{-}OH_2 \underset{k_d^*}{\overset{k_f}{\rightleftharpoons}} CBM\text{-}SO_3^{2-} + H_3O^+ \quad (5)$$

From the equilibrium data of Pratt and coworkers[11] and assuming $pK_a^{HSO_3} = 7.0$ the equilibrium quotient for eq 5 ($\mu \simeq 0.35$ *M*, room temperature) can be calculated.

$$K = \frac{(CBM\text{-}SO_3)(H_3O^+)}{(CBM\text{-}OH_2)(HSO_3^-)} = 2.2 \quad (6)$$

The sulfito complex is sufficiently stable at pH 5.0 to allow one to follow the anation reaction under conditions where (CBM-OH_2) ≫ (L), with (CBM-OH_2) = 0.6–1.5 × 10^{-4} *M*. Values of k_f so obtained (determined using a Cary Model 14 spectrophotometer equipped with a 0–0.1 OD slidewire) were within experimental error of those determined using excess ligand and the stopped-flow method. Assuming eq 5 represents the only substitution mechanism at pH 5, k_d^* (a second-order rate constant associated with an acid-catalyzed aquation) may be calculated from *K* and k_f.

No significant change in the HSO_3^- anation rate was found between pH 3.7 and 5.8, indicating that under these conditions kinetic paths involving H_2SO_3 or SO_3^{2-} are unimportant.[14] The product spectrum was also pH independent in this range, consistent with the stoichiometry of eq 5. Acid independence up to pH 6

(11) F. A. Firth, H. A. O. Hill, J. M. Pratt, R. G. Thorp, and R. J. P. Williams, *J. Chem. Soc. A*, 381 (1969).

(12) The cobalamin molecule with the cobalt–benzimidazole bond intact is represented by

It will often be abbreviated as CBM–. The presence of a coordinated water in B_{12a} has not been proven. However, indirect evidence supports six-coordination,[13] and a cobalt-bound water has been found in the cobyric acid derivative.[4]

(13) G. C. Hayward, H. A. O. Hill, J. M. Pratt, N. J. Vanston, and R. J. P. Williams, *J. Chem. Soc.*, 6485 (1965).

(14) The pK_a values at 25° for sulfurous acid and bisulfite ion are 1.8 and 7.0.

implies an upper limit of $\simeq 2 \times 10^2\ M^{-1}\ sec^{-1}$ for the SO_3^{2-} anation rate constant. From the equilibrium quotient[11] for the reaction $SO_3^{-2} + CBM\text{-}OH_2 \rightleftharpoons CBM\text{-}SO_3^{-2} + H_2O$, a limiting k_d value of $< 1 \times 10^{-5}$ sec^{-1} is implied for the aquation of the sulfito complex.

Randall and Alberty[8] have already reported on the cyanate reaction, and therefore this system was not investigated in detail. The cyanate anation reactions were studied at a single ligand concentration (0.012 M) and eq 4 was assumed in calculating the apparent second-order rate constants (k_d was determined independently as described below). The pH of the anation reactions was 5.3, which is in the range where the principal reaction pathway involves aquocobalamin and the unprotonated ligand.[8] When the pH dependence is considered (eq 18, ref 8), the apparent rate constant at pH 5.3 (450 $M^{-1}\ sec^{-1}$) may be corrected to a value of 470 $M^{-1}\ sec^{-1}$ for the binding of unprotonated ligand to aquocobalamin.

The binding of azide was followed at pH 5.4. The apparent rate constant of $1.0 \times 10^3\ M^{-1}\ sec^{-1}$ determined at this pH was corrected for hydrogen ion dependence using eq 8 of ref 8, giving $k_f = 1.18 \times 10^3\ M^{-1}\ sec^{-1}$.

Aquocobalamin (0.86×10^{-5} M) was titrated with azide ($0.9\text{–}20 \times 10^{-5}$ M) at pH 5.9. The difference spectrum ($OD_{CBM\text{-}N_3} - OD_{CBM\text{-}OH_2}$) exhibited a positive maximum at 367 nm, a negative maximum at 350 nm, and an isosbestic point at 357 nm. The quantity $\Delta OD_{367-350}$ was determined as a function of total azide concentration, and the data were fitted directly to eq 7 using an iterative procedure and an electronic calculator.

$$\Delta OD_{367-350} = \frac{(CBM\text{-}OH_2)_0}{1 + 1/K_{app}(N_3^-)_0}\Delta\epsilon_{367-350} \qquad (7)$$

In deriving eq 7 it is assumed that (1) the stoichiometry is 1:1, and (2) ligand is present in large excess.

Since $(CBM\text{-}OH_2)_0$ was not negligible relative to $(N_3^-)_0$, it was necessary to include in the program a correction to equilibrium azide concentrations. A weighing factor of unity was assumed in the least-squares analysis, which is appropriate when the error in ΔOD is constant. The results of the titration were $K_{app} = 5.2 \pm 0.3 \times 10^4\ M^{-1}$, $\Delta\epsilon_{367-350} = 1.34 \pm 0.02 \times 10^4$ OD $M^{-1}\ cm^{-1}$ (pH 5.9, 0.10 M MES buffer, 0.25 M KNO_3, 25.8 ± 0.5°). When the concentration of HN_3 and CBM–OH is taken into account, we calculate $K = 5.6 \pm 0.3 \times 10^4\ M^{-1}$ for the binding of N_3^- to $CBM\text{-}OH_2$ (eq 15, ref 8). Using ΔOD_{367} and ΔOD_{350} as dependent variables, the following parameters were obtained: $K = 5.8 \pm 0.2 \times 10^4\ M^{-1}$ and $\Delta\epsilon_{367} = 7.61 \pm 0.01 \times 10^3$ OD $M^{-1}\ cm^{-1}$; $K = 5.2 \pm 0.6 \times 10^4\ M^{-1}$ and $\Delta\epsilon_{350} = 5.8 \pm 0.2 \times 10^3$ OD $M^{-1}\ cm^{-1}$.

The above equilibrium constants may be compared with the values of $K = 5.6 \times 10^4\ M^{-1}$ ($\mu = 0.054$, 25°)[8] determined directly by titration between pH 2 and 8, and $K = 7.2 \times 10^4\ M^{-1}$ ($\mu = 0.5$, room temperature), determined indirectly by competition with OH^- at pH 9.9.[11]

The bromide reaction was too fast to be monitored with the stopped-flow method, but could be conveniently followed using a temperature-jump technique. The concentration dependence of the reciprocal relaxation time for a bimolecular process is given by[15]

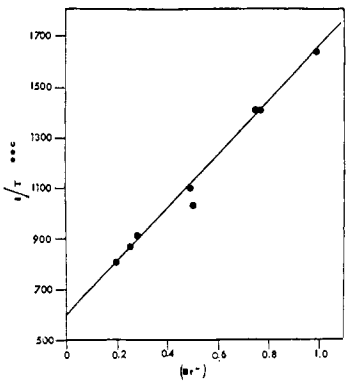

Figure 3. Ligand dependence of the Br^- reaction.

$$\tau^{-1} = k_f[\overline{(CBM\text{-}OH_2)} + \overline{(L)}] + k_d \qquad (8a)$$

If $(L)_0 \gg (CBM\text{-}OH_2)_0$

$$\tau^{-1} = k_f(L)_0 + k_d \qquad (8b)$$

The bromide data are presented in Figure 3.

As noted above, most plots of k_{app} *vs.* (L) do not have measurable intercepts. Although k_d could be obtained by extending the rate measurements to lower ligand concentrations, a more convenient means of determining aquation rates is to shift equilibrium 1 completely to the left by the addition of base

$$H_2O + CBM\text{-}L \xrightarrow[\text{slow}]{k_d} CBM\text{-}OH_2 + L \xrightarrow[\text{fast}]{OH^-} CBM\text{-}OH + L \qquad (9)$$

where the second step is a rapid proton-transfer reaction. This method assumes that under the conditions employed here, hydroxide-dependent hydrolysis paths are unimportant. This assumption is supported by the fact that the rate of hydrolysis of the thiocyanato complex is independent of (OH^-) (2×10^{-3}–2×10^{-2} M) and (SCN^-) (0.10–0.41 M).[16] In addition, the rate of hydrolysis of the azide complex is independent of (OH^-) in the range 0.02–0.10 M (determined at $(N_3^-) = 0.01$ M). Moreover, the experimental rate constants are in satisfactory agreement with the values calculated from the stability constants and anation rate constants.

Some experiments were performed to determine the effect of changes in ionic medium on the reaction rates. Decreasing μ from 0.5 to 0.05 M increased the SCN^- anation rate by a factor of 2.5. The rate of $CBM\text{-}S_2O_3$ formation and dissociation is not appreciably changed in going from 0.5 M $NaClO_4$ to 0.5 M KNO_3.

Dissociation and second-order formation rate constants at 25° are summarized in Table I, along with the kinetic and thermodynamic stability constants. Rate constants were also determined at temperatures other than 25° (Figures 4 and 5). With the exception of I^- (Figure 2), formation rate constants at these other temperatures were calculated from pseudo-first-order constants determined at a single ligand concentration. In

(15) M. Eigen and L. DeMaeyer in "Techniques of Organic Chemistry," Vol. III, Part II, S. L. Friess, J. Lewis, and A. Weissberger, Ed., Interscience, New York, N. Y., 1963.

(16) The aquation of the SCN^- complex is biphasic, in qualitative agreement with the existence of an equilibrium mixture of isomers.[10] The data reported here refer to the aquation of the more stable species.

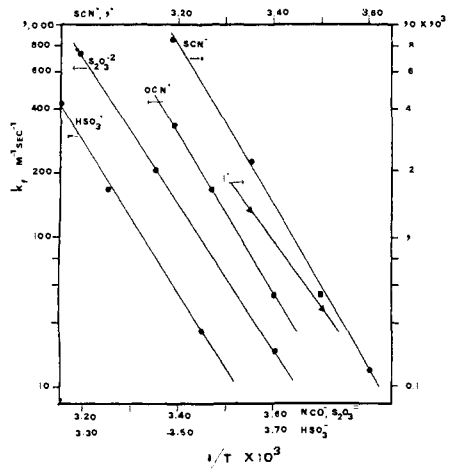

Figure 4. Arrhenius plots for the anation reactions.

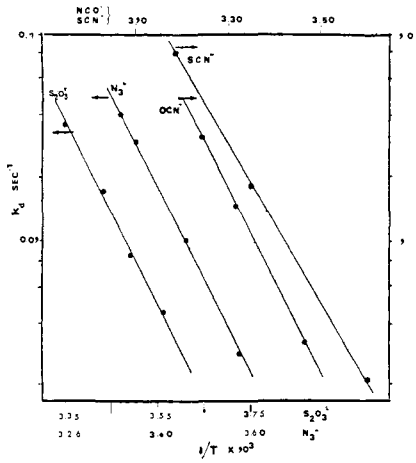

Figure 5. Arrhenius plots for the aquation reactions.

some cases a small correction for the back-reaction was necessary. Activation parameters are given in Table II.

Table I. Summary of Rate[a] and Stability Constants (Temp = 25.5 ± 0.2°; μ = 0.5 M, ClO_4^- Medium)

L	$10^{-3}k_f$, M^{-1} sec^{-1}	k_d, sec^{-1}	K_{kinet}, M^{-1}	K_{thermo},[b] M^{-1}
SCN^-	2.3	1.8	1.3×10^3	1.2×10^3
I^- [c]	1.4	3.5×10^1	3.4×10^1	3.2×10^1
Br^- [d]	1.0	5.9×10^2	1.7	1.9
N_3^-	1.2	2.9×10^{-2} [g]	4.1×10^4	5.6×10^4 [e]
NCO^- [h]	0.47	1.1	4.3×10^2	5.3×10^2
$S_2O_3^{2-}$	0.20	3.5×10^{-2}	5.8×10^3	7.3×10^3
HSO_3^-	0.17	(~80 M^{-1} sec^{-1})[i]		~2.2[f]
SO_3^{2-}	≲0.2	≲1×10^{-5}		2.2×10^7 [f]

[a] Errors in rate constants are about ±10%, except for the aquation of the iodo complex, where the error is closer to ±20%. [b] Taken from ref 6, unless otherwise noted. Ionic strength = 0.5 M KNO_3 (except for $S_2O_3^{2-}$, where medium was $NaClO_4$) and ambient temperature. [c] Supporting electrolyte was KNO_3. [d] 26 ± 1°, μ = 1.0 M, supporting electrolyte was KNO_3. [e] This study. [f] Taken or estimated from data in ref 11. [g] 25.0 ± 0.2°. [h] Interpolated from the rate constants determined at temperatures other than 25° See text.

(b) Temperature-Jump Experiments at High Ligand Concentrations. Some temperature-jump experiments were performed at ligand concentrations where essen-

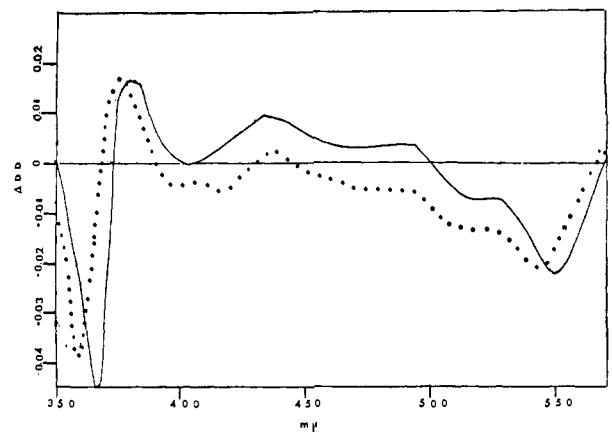

Figure 6. Difference (33°/9°) spectra with the sample cell at the higher temperature; complex concentration = 6×10^{-5} M, 1-cm cells. The accuracy of ΔOD is about ±0.002: (—) sulfitocobalamin, 0.020 M $NaHSO_3$, 0.50 M $NaClO_4$, pH 6.0; (···) azidocobalamin, 0.012 M NaN_3, 0.50 M $NaClO_4$, pH 6.6.

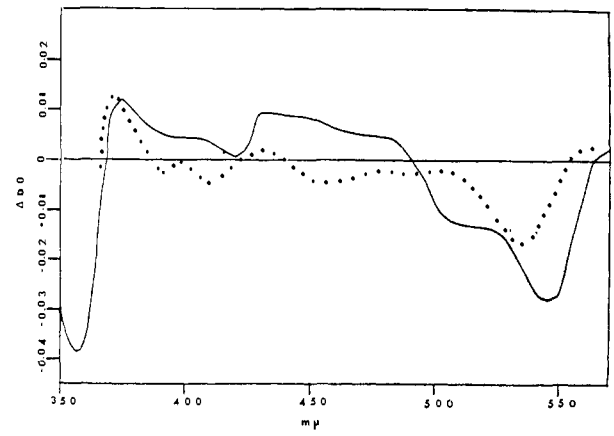

Figure 7. Temperature difference spectra (*cf.* caption to Figure 6); (—) hydroxocobalamin, 0.20 M NaOH; (···) aquocobalamin, pH 4.5 ($HClO_4$), no added electrolyte.

tially all of the cobalamin existed as CBM–L (L = SCN^-, SO_3^{2-}, $S_2O_3^{2-}$, N_3^-, and OH^-). In every case a rapid change in absorption (complete in ≲20 μsec) was observed. From eq 8, the rate constants of Table I, and the ligand concentrations employed, it could be shown that these rates were too large to be accounted for merely by the perturbation of equilibrium 1.

Table II. Activation Parameters for Some Cobalamin Anation and Aquation Reactions[a]

L	$\Delta H_f^{\pm}$, kcal/mol	$\Delta S_f^{\pm}$, eu	$\Delta H_d^{\pm}$, kcal/mol	$\Delta S_d^{\pm}$, eu
SCN^-	17.1 ± 0.4	+14 ± 1	17.2 ± 0.5	+0.4 ± 2
I^-	14 ± 2	+3 ± 8		
N_3^-			20.5 ± 0.5	+3 ± 2
OCN^-	15.7 ± 0.6	+6 ± 2	18.3 ± 0.4	+3 ± 2
$S_2O_3^{2-}$	15.0 ± 0.7	+2 ± 2	19.2 ± 0.5	−0.7 ± 2
HSO_3^-	15.6 ± 1.2	+4 ± 4		

[a] The activation enthalpy and entropy were calculated from the Arrhenius activation energy and frequency factor using the relationships $E_a = \Delta H^{\pm} + RT$ and $A = (ekT/h)\exp(\Delta S^{\pm}/R)$.

The temperature-dependent changes in absorption have been verified by temperature-difference spectra (Figures 6 and 7). In the case of N_3^-, the ligand con-

centration and pH were varied. The difference spectrum of CBM-N_3 was independent of N_3^- in the range 0.012–0.12 M, and of pH in the range 6.0–8.9. It should be noted that for HSO_3^- and OH^-, the ligand concentration was sufficient to place less than 0.01% of the cobalamin in the aquo form, precluding the possibility that the observed changes in absorption arise from the anation reactions.

Aquocobalamin also exhibited an "immeasurably fast" relaxation effect. The ΔOD of the temperature difference spectrum (Figure 7) was proportional to (CBM-OH_2) in the range 0.5–1.6 × 10^{-4} M. That the changes in absorbance are not due to a dimerization is indicated by the fact that Beer's law is obeyed up to at least 2.0 × 10^{-4} M.

An attempt was made to follow the CBM-S_2O_3 effect using the field dissociation method, which has an ~20-fold larger resolution time than the temperature-jump technique.[17] No relaxation could be detected, however, presumably because the phenomenon in question does not involve a substantial change in charge distribution.

A relaxation amplitude analysis suggested that the changes in OD are associated with an intramolecular process. Table III gives the relaxation amplitudes for

Table III. Amplitude of the Fast Relaxation Effect Observed in the SCN^-–CBM-OH_2 Reaction (λ 568 mμ, pH 4.0–5.9, μ = 0.50 M Perchlorate Medium, $Temp_0$ = 13°, ΔT = 12°)

(SCN^-)	$(Cobalamin)_{tot}$ × 10^4, M	$\overline{(CBM\text{-}L)}$ × 10^4, M	ΔI, mV	$\Delta OD/\overline{(CBM\text{-}L)}$[a,d,e]
0.50	1.9	1.9	8.0	38
0.50	0.85	0.85	5.0	37
0.010	1.9	1.7	7.0	35
0.0043	2.9	2.4	10.0	33[b]
0.0020	2.9	2.0	10.0	35[c]

[a] $\Delta OD = \Delta I/2.3(I_\infty)$. [b] pH 5.9. [c] pH 4.0. [d] The amplitude of the OD change associated with aquocobalamin is negligible at the wavelength employed here. [e] Thermal expansion of the solvent would account for less than 10% of the observed ΔOD.

the thiocyanato complex at different reagent concentrations. Within the limits imposed by the experimental uncertainty in ΔOD, the quantity ΔOD/CBM-L is independent of $(CBM\text{-}OH_2)_0$, (SCN^-), (ClO_4^-), and pH. Following the development of Eigen and DeMaeyer,[15] the relaxation amplitude for an apparent one-step reaction may be expressed as

$$\Delta OD = \Delta\epsilon\Gamma(\Delta H/RT^2)\,\Delta T \qquad (10)$$

where $\Delta\epsilon$ = apparent extinction coefficient, ΔH = reaction enthalpy, and ΔT = temperature change.

The Γ factor (M) is defined by the stoichiometry of the reaction. If the observed transformation is intramolecular, for example

$$CBM\text{-}L' \rightleftharpoons CBM\text{-}L'' \qquad (11)$$

Γ is given by

$$\Gamma = \overline{CBM\text{-}L}/(1 + K)(1 + 1/K) \qquad (12)$$

$$\overline{CBM\text{-}L} = \overline{CBM\text{-}L'} + \overline{CBM\text{-}L''} \qquad (13)$$

$$K = \overline{CBM\text{-}L''}/\overline{CBM\text{-}L'} \qquad (14)$$

(17) We are grateful to Dr. George Ilgenfritz for making these measurements.

and the other quantities in eq 10 are

$$\Delta H = H_{CBM\text{-}L''} - H_{CBM\text{-}L'} \qquad (15)$$

$$\Delta\epsilon = \epsilon_{CBM\text{-}L''} - \epsilon_{CBM\text{-}L'} \qquad (16)$$

Rearrangement of eq 10 shows that for an intramolecular process ΔOD/CBM-L would be concentration independent.

$$\Delta OD/CBM\text{-}L = \frac{\Delta\epsilon}{(1 + K)(1 + 1/K)}\frac{\Delta H}{RT^2}\Delta T \qquad (17a)$$

However, a discrete chemical change as implied in eq 11 is not a unique interpretation of the amplitude data. In particular, the fast OD changes could arise from temperature-dependent extinction coefficients. In this case the quantity $\Delta OD/\overline{CBM\text{-}L}$ would again be concentration independent,

$$\Delta OD/CBM\text{-}L = \left(\frac{\partial\epsilon_{CBM\text{-}L}}{\partial T}\right)_P \Delta T \qquad (17b)$$

Here CBM-L represents a single chemical component. At wavelengths where $\epsilon_{CBM\text{-}OH_2}$ is not negligible, the proportionality constant becomes $(\partial(\epsilon_{CBM\text{-}L} - \epsilon_{CBM\text{-}OH_2})/\partial T)_P$.

Discussion

The data of Table I show that k_f is relatively independent of the nature of the incoming group. Although the stability constants vary by 2 × 10^4 the formation rates are nearly the same order of magnitude. In addition, anation activation energies are very similar. The differences in rates which do exist cannot be correlated in a simple way with the stabilities of the complexes. This rather weak response to variations in ligand nucleophilicity is even more remarkable when one includes estimates of the CN^- parameters (k_f = 1500 M^{-1} sec^{-1}, K = 10^{12} M^{-1}).[8] A reasonable conclusion is that bond formation does not play an important role in the anation reactions and that the flux is limited by the dissociative loss of water, as found for numerous other aquo complexes.[2,3]

An estimate of the degree of bond breaking in the aquation reactions involving I⁻ ligands has been made from a "linear free energy relationship."[18,19] Figure 8 shows a reasonably linear relation between $\Delta\Delta F^{\ddagger}$ and $\Delta\Delta F°$, indicating a common rate-determining step for these reactions. The slope of the line in Figure 8 is 1.0. This suggests a transition state characterized by well-advanced, if not complete, bond dissociation.[18,19] Using Langford's approach,[19] one can also speculate as to the role of the entering water molecule. In the present case, where the departing ligand closely resembles the free anion, the entering group will be strongly bound (*i.e.*, the transition state will resemble CBM-OH_2) only if the overall reaction is highly endothermic. For the ligands of Table II, $\Delta F^{\ddagger}_d$ is 17–19 kcal/mol, whereas the corresponding $\Delta F°_d$ values are 0.3–6 kcal/mol. Relative to kinetic activation, the overall processes are not far from being "thermally balanced," implying that both water and anion are at best loosely bound in the transition state. Also noteworthy in this connection is the absence of ligand as-

(18) J. E. Leffler and E. Grunwald, "Rates and Equilibria of Organic Reactions," Wiley, New York, N. Y., 1963, p 156.
(19) C. L. Langford, *Inorg. Chem.*, 4, 265 (1965).

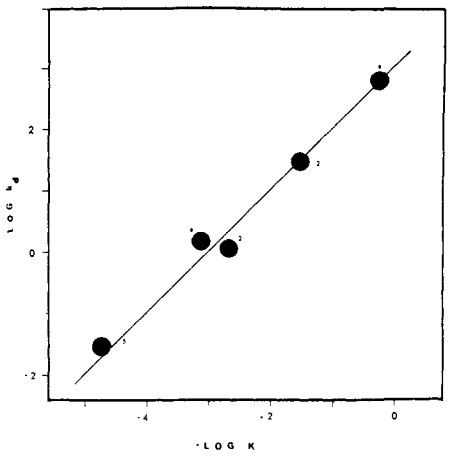

Figure 8. Free energy plot for cobalamin aquation reactions (25°) involving mononegative ligands: 1, Br^-; 2, I^-; 3, NCO^-; 4, SCN^-; 5, N_3^-

sistance by hydroxide ion, at least at the concentrations employed in the present study.

The above observations are consistent with two different mechanisms in which unimolecular dissociation of the leaving group plays a paramount role.[2,3,20] In one mechanism the reaction proceeds *via* an interchange between ligand and water in a rapidly formed outer-sphere complex.

$$\text{CBM-OH}_2 + \text{L} \overset{K_0}{\rightleftharpoons} \text{CBM-OH}_2\cdot\text{L} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{CBM-L} + \text{H}_2\text{O} \quad (18)$$

If $(\text{CBM-OH}_2\cdot\text{L}) \ll (\text{CBM-OH}_2), (\text{CMB-L})$

$$k_f = K_0 k_1 \quad (19)$$

$$k_d = k_{-1} \quad (20)$$

The other mechanism is of the limiting SN1 type, characterized by an intermediate of reduced coordination number.

$$\text{CBM-OH}_2 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \text{CBM}^- + \text{H}_2\text{O} \quad (21)$$

$$\text{CBM}^- + \text{L} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \text{CBM-L} \quad (22)$$

If the intermediate is in a steady state

$$k_f = k_2 k_3/(k_{-2} + (\text{L})k_3) \quad (23)$$

$$k_d = k_{-2} k_{-3}/(k_{-2} + (\text{L})k_3) \quad (24)$$

when $k_{-2} \gg k_3(\text{L})$

$$k_f = (k_2/k_{-2})k_3 \quad (25)$$

$$k_d = k_{-3} \quad (26)$$

It is of interest that Randall and Alberty[7,8] have concluded that the kinetics of these systems cannot be accounted for in terms of a rate-limiting, unimolecular loss of water followed by a very rapid binding of ligand. Assuming (1) step 22 is very rapid relative to step 21, and (2) the relaxation time associated with eq 22 is not detectable,[21] these authors derive the following expression for the experimentally observed relaxation time of a limiting SN1 mechanism.

$$1/\tau = k_{-2} + k_{+2}/(1 + \bar{\text{L}}/K_{\text{L}}) \quad (27)$$

$$K_{\text{L}} = \overline{(\text{CBM})}\bar{\text{L}}/\overline{\text{CBM-L}} \quad (28)$$

Since in all cases plots of $1/\tau$ *vs.* $\bar{\text{L}}$ linearly increase, it was concluded[8] that unimolecular release of water from aquocobalamins is not the rate-limiting step.[22]

Implicit in the above treatment is the assumption that the equilibrium concentration of CBM- is comparable to that of CBM-OH_2 and CBM-L. If, however, the five-coordinated intermediate exists at relatively low, steady-state concentrations, the two relaxation times would coalesce and result in a single time constant. Assuming $\bar{\text{L}} \gg \overline{\text{CBM-OH}_2}$, the "steady-state" reciprocal relaxation time is given by eq 8b, where the apparent rate constants k_f and k_d are given in eq 23 and 24.

Aquocobalamin is astonishingly labile for a diamagnetic cobalt(III) complex.[7,8,13] Many CBM-L aquation rates are also quite fast, even though cobalamins form relatively stable complexes. Two exceptions are CBM-SO_3 and CBM-CN, which are extremely stable both kinetically and thermodynamically. The reactivity of aquocobalamin arises largely from anomalously low activation energies, since $\Delta H^{\pm}$ for the replacement of water in Co(III) complexes is usually *ca.* 10 kcal higher than observed here.[2,3] Some qualitative observations suggest that a kinetic trans effect by benzimidazole could be only in part responsible for the cobalamin lability. Derivatives containing no benzimidazole side chain are known to undergo substitution very rapidly,[13] and at low pH benzimidazole itself is readily displaced from many cobalamins.[6] Also pertinent is the unusual reactivity of cobalt(III) hematoporphyrin.[23] It would appear that some property common to both the corrin and porphyrin ring systems can dramatically alter the lability of axial ligands, but the nature and origin of the effect remain obscure.

It has been shown that the model cobalamin complexes, nitroaquobis(dimethylglyoximato)cobalt(III) and iodoaquobis(dimethylglyoximato)cobalt(III), are substitution inert.[24] Although the bisdimethylglyoximes (or "cobaloximes") exhibit some of the unique properties of cobalamins, such as stable Co^{III}-S and Co^{III}-C bonds,[25-27] the cobalamins are up to 10^7 more labile. The reasonable suggestion has been made that the difference in reactivity between cobalt(III) hematoporphyrin and the cobaloximes cannot be accounted for by steric factors, owing to similarities in charge and geometry.[23] The same would appear to hold for the cobalamins. However, a comparison of the cobalamin anation activation parameters with those found for the bis(dimethylglyoximato)cobalt(III) complexes of ref 24 shows that the rate differences can be attributed in

(20) C. H. Langford and H. B. Gray, "Ligand Substitution Processes," W. A. Benjamin, New York, N. Y., 1966.

(21) It has been stated in ref 7 that when step 22 proceeds so rapidly that it remains at equilibrium with respect to step 21, only one relaxation will be observed. Although this is true if $(\epsilon_{\text{CBM}^-} - \epsilon_{\text{CBM-L}}) = 0$ or $(H_{\text{CBM}^-} - H_{\text{CBM-L}}) = 0$, it is not true in the general case.

(22) The same conclusion is reached in the present work; the ligand independence of k_f and k_d requires that $k_{-2} \gg k_3(\text{L})$.

(23) E. B. Fleischer, S. Jacobs, and L. Mestichelli, *J. Amer. Chem. Soc.*, **90**, 2527 (1968).

(24) D. N. Hague and J. Halpern, *Inorg. Chem.*, **6**, 2059 (1967).

(25) G. N. Schrauzer and R. J. Windgassen, *J. Amer. Chem. Soc.*, **88**, 3738 (1966).

(26) G. N. Schrauzer and R. J. Windgassen, *ibid.*, **89**, 143 (1967).

(27) A. V. Ablov and I. D. Samus, *Dokl. Akad. Nauk SSSR*, **146**, 1071 (1962).

large part to entropy effects.[28] If it were not for sizable negative entropies of activation, the cobaloximes would in fact be very labile compared to many "normal" aquocobalt(III) complexes.[2,3] In view of the striking differences in activation entropies, it seems worthwhile to ask whether the cobaloximes and cobalamins might react *via* different mechanisms. Large negative activation entropies and rather small activation enthalpies can be indicative of significant bond formation in the activated complex.[3] Obviously, however, the meaning of the present observations will become clear only after more experimental data are available.

The fast OD changes observed under conditions where essentially all of the cobalamin is saturated with ligand clearly do not arise from the perturbation of equilibrium 1. Furthermore, the ligand-, pH-, and supporting-electrolyte-independent amplitudes observed for L = SCN^-, the ligand- and pH-independent difference spectrum for L = N_3^-, and the known stoichiometry of the systems under consideration[6-8,11] argue against ion-pair formation, proton-transfer reactions, or displacement of the benzimidazole group by a second mole of ligand.

Because of its intramolecular nature and very high rate, it is tempting to attribute the phenomenon to a subtle structural change in the corrin ring. It is of interest that porphyrins and corrinoids are known to be flexible and to possess regions of nonplanarity.[4,5,29,30] The difficulties inherent in terming a temperature-dependent structural change in the corrin ring as (1) a change in conformation, (2) a change in the distribution over vibrational states, or (3) an electronic effect have recently been discussed by Firth, *et al.*,[31] who have also observed changes in cobalamin spectra on varying the temperature. Although it is not certain that the process in question can be described by a single category, the temperature-difference spectra would seem to favor the second classification;[31] a comparison of the overall features of Figures 6 and 7 with the absorption spectra of the individual complexes indicates that a lowering of temperature results for the most part only in an increase in the sharpness and intensity of the main absorption bands.[32]

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(28) At least two DMG aquation reactions also exhibit large negative entropies relative to many "normal" cobalt(III) complexes. Using rate data from ref 24 activation parameters for the aquation of *trans*-$Co(DH)_2(NO_2)Cl^-$ and *trans*-$Co(DH)_2(NO_2)Br^-$ have been calculated. For the chloro complex, $\Delta H^{\ddagger} = 16.7$ kcal/mol, $\Delta S^{\ddagger} = -21$ eu; and for the bromo complex, $\Delta H^{\ddagger} = 18.3$ kcal/mol, $\Delta S^{\ddagger} = -16$ eu.

(29) J. L. Hoard in "Hemes and Hemoproteins," B. Chance, R. W. Estabrook, and T. Yonetani, Ed., Academic Press, New York, N. Y., 1966, pp 9–23.

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(32) We are grateful to Dr. J. M. Pratt for a communication on this point.

Kinetics of Ligand Exchange with Nickel(II) Triglycine

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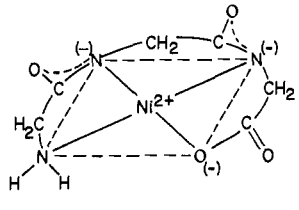
Abstract: Steric hindrance, chelation, and donor ability are important factors in the reactivity of ligands with $NiH_{-2}L^-$ (where L^- is the triglycinate ion and protons are ionized from the two peptide nitrogens coordinated to nickel). Ligands can react by a nucleophilic mechanism similar to that proposed for the analogous copper complex, $CuH_{-2}L^-$. Ethylenediamine and polyamine ligands have second-order rate constants of $(1.2\text{–}1.7) \times 10^4\ M^{-1}\ sec^{-1}$ at 25° and are most effective in breaking up the square-planar $NiH_{-2}L$ complex. The diamines and polyamines are (2–3) $\times 10^3$ times more reactive than ammonia. Addition of *N*-methyl groups to ethylenediamine has relatively little kinetic effect until both nitrogens are tertiary and then the reactivity decreases sharply. Glycinate ion and *N,N*-dimethylglycinate ion have comparable reactivities but are much slower than the sterically unhindered diamines. In order for a ligand to be an effective nucleophile one donor group must be able to coordinate to nickel in the plane of the nickel–N (peptide) bonds. The order of reactivity is: diamines ≫ aminocarboxylates ≫ monodentate ligands ($NH_3 \gg CH_3COO^-$). Trimethylamine is unreactive.

Triglycine (glycylglycylglycine) forms a yellow, square-planar complex with nickel(II) in which two protons are ionized from the peptide nitrogens and the nitrogen atoms are coordinated to nickel.[1-3] This complex, $NiH_{-2}L^-$ (depicted in structure I), reacts with acids[4] or with ligands to give blue, octahedral nickel(II)



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