Effects of Surfactants on Cobalamin Dependent Methyl Transfer. Influence of Aqueous and Reversed Micelles on the Interaction of Mercuric Ion with Methylcobalamin

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Abstract: Interaction of methylcobalamin (I) with $Hg(OAc)_2$ in water involves two consecutive steps: the initial rapid reversible build-up of the "base-off" methylcobalamin mercuric acetate complex (II) which is followed by the slower formation of aquocobalamin (III). Rate constants for the formation, k_1^{app}, and decomposition, k_{-1}^{app}, of II and that for the formation of III, k_2^{app}, in water at 25.0 °C and at pH 4.50 have been determined to be 4000 M^{-1} s^{-1}, 54 M^{-1} s^{-1} and 380 M^{-1} s^{-1}, respectively. Addition of excess chloride or bromide ion simplifies the overall reaction by eliminating the observable formation of II. In these cases, the attacking species are the mercuric complexes of chloride and bromide ions. Rate constants for the attack of $HgCl_2$ on I, k_4^{app}, have been determined in the pH 2.0–5.5 region. The data yielded the pH independent rate constants k_4 = 4.0 M^{-1} s^{-1} at 25.0 °C. Addition of sodium dodecyl sulfate, SDS, causes a marked decrease in k_4^{app} in water. The inhibition is the consequence of the alteration of the pK_a for dissociation constant of I to form the "base-off" methylcobalamin (IV) from 2.63–2.73 in water to 5.67 in micellar SDS and of the differential rates in micellar and bulk aqueous phases. The pH dependence of k_4^{app} in 0.10 M SDS parallels that in water, but is shifted to 3 pH units higher. The pH independent rate constants for the interaction of $Hg(OAc)_2$ and $HgCl_2$ in 0.10 M SDS at 25.0 °C have been calculated to be k_2 = 2.33 M^{-1} s^{-1} and k_4 = 0.11 M^{-1}. Thus the micellar inhibition for these reactions are 160- and 36-fold, respectively. Aqueous micellar hexadecyltrimethylammonium bromide, CTABr, completely stops the methyl transfer. Reaction of I with $Hg(OAc)_2$ in dodecylammonium propionate solubilized water pools in benzene is ca. 18 000-fold slower than that in bulk water. Significance of these results are discussed.

The importance of vitamin B_{12} mediated transfer of alkyl groups in vivo has prompted the numerous investigations of this process in dilute aqueous solutions.[2-5] Biological alkyl transfer occurs, however, not in dilute aqueous solutions, but in hydrophobic environments. Indeed vitamin B_{12} and its derivatives are all tightly bound to proteins.[2,6,7] Surfactant aggregates in water, aqueous micelles, or those in nonpolar solvents, reversed micelles, have been profitably employed to mimic the infinitely more complex biochemical media.[8,9] Investigations of reactions in micelles can be considered to provide the necessary bridge between reactions of uncomplexed molecules in water and those of enzyme-catalyzed ones in vivo. Pronounced effects of aqueous micellar hexadecyltrimethylammonium bromide (CTABr) and sodium dodecyl sulfate (SDS) have been observed on the rate constants for the interaction of vitamin B_{12a} with *N*-alkanoyl-D,L-cysteines.[10] These effects have been rationalized in terms of the determined partitions of the reactants between the bulk water and the aqueous micellar pseudophase.[10] More dramatic effects have been found on the rate constants for the attack of glycine, imidazole, and sodium azide on vitamin B_{12a} in reversed micelles.[11] These micellar effects were specific and could not be accounted for in terms of partitioning the reactants between the surfactant entrapped water pools and the bulk apolar solvent.[9,11] Significantly, the effective polarity of the environment of vitamin B_{12} in surfactant solubilized water pools in benzene could be altered from that resembling water to that resembling benzene by varying the concentration of the solubilized water. Maximum rate enhancements were observed in the least polar reversed micelles provided for by solubilizing minimum amounts of water.[9,11]

The present work represents our first examination of micellar effects on methyl transfer involving vitamin B_{12}. We chose the reaction between methylcobalamin and mercuric ion. This reaction has been extensively investigated previously in water.[12-20] At the onset of our work we had assumed it, therefore, to be free of mechanistic ambiguities. This assumption proved to be overly optimistic. In addition to reporting micellar effects on the rates of methyl transfer from methylcobalamin to mercury ion we offer additional insight to the mechanism of this reaction in water.

Experimental Section

Vitamin B_{12a}, aquocobalamin (Merck), was used as received. Methylcobalamin was prepared by the sodium borohydride reduction of aquocobalamin followed by oxidative addition of methyl iodide.[21,22] Typically, solutions of aquocobalamin (250 mg in 50 mL of H_2O in the presence of 5 drops of 0.10 M aqueous $CuSO_4$) and $NaBH_4$ (0.2 g in 5.0 mL of H_2O) were separately purged by purified nitrogen in closed vessels through rubber septa for 2 h. A hypodermic needle punched through just below the rubber septa provided the vent. Subsequent to deoxygenations the two solutions were mixed, and allowed to react for 30 min. The reaction vessel was then wrapped with aluminum foil to exclude all light. CH_3I (1 mL) was injected into the solution and allowed to react for 10 min. Nitrogen purging continued for the entire reaction time. The reaction was stopped by the addition of 2.5 mL of acetone. All subsequent operations were carried out in the dark. The reaction mixture was poured into a separatory funnel containing 60 mL of 90% aqueous phenol. The methylcobalamin partitioned into the phenol phase. The aqueous phase was discarded and the phenol phase was washed twice with 25 mL of H_2O. Ether (160 mL) and 40 mL of acetone were added to the phenol solution. The organic solution was then extracted three times each with 35 mL of water. All the red color (methylcobalamin) was removed to the aqueous layer. The combined aqueous layer was washed twice each with 50 mL of ether. The ether washings were discarded. Last traces of ether were removed from the aqueous solution by nitrogen bubbling. The methylcobalamin solution was purified by passing through a 3 in. × 22 mm OH^--DEAE-cellulose column, followed by a 6 in. × 22 mm H^+-CM-cellulose column. The eluent was concentrated to a few milliliters in a rotary evaporator at <30 °C. A few drops of acetone were added just below turbidity and crystals were formed in the refrigerated solution overnight. The liquor was decanted off and the deep red crystals were washed with minimal amounts of acetone and ether. The freshly prepared methylcobalamin was dried in a vacuum desiccator over $CaSO_4$, yield 90%. The purity of methylcobalamin was established by spectrophotometry and kinetics. The determined rate constant for the interaction of K_2PdCl_4 with methylcobalamin in water at pH 5.00 and 25.0 °C, k_1^{app} = 7.8 × 10^{-1} M^{-1} s^{-1} (calculated from the following first-order rate constants: k_Ψ = 8.2 × 10^{-4} s^{-1},

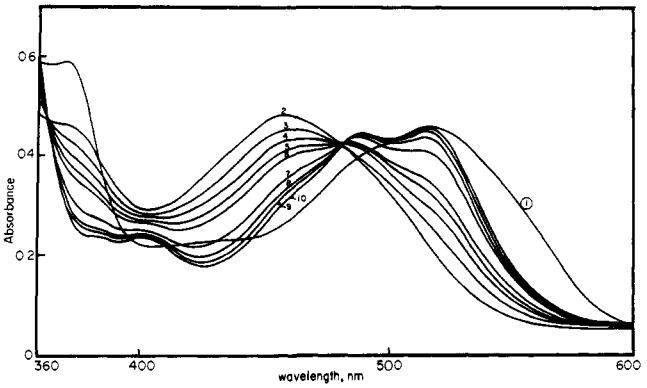


Figure 1. Absorption spectra of 5.0×10^{-5} M methylcobalamin in 0.10 M sodium acetate-acetic acid buffer at pH 4.50 in the presence of 1.00×10^{-4} M $Hg(OAc)_2$ at 25.0 °C. At time zero (1), 0.5 (2), 4 (3), 7.5 (4), 11 (5), 14.5 (6), 21 (7), 28 (8), 35 (9), and 180 (10) min subsequent to mixing the reagents.

Table I. Interaction of Methylcobalamin with $Hg(OAc)_2$ in Water at 25.0 °C[a]

$10^4[Hg(OAc)_2]$, M	k_Ψ^{fast}, s^{-1}	k_Ψ^{slow}, s^{-1}
5.0	2.25	
10.0		0.65
15.0	7.60	0.84
20.0	10.70	1.21
25.0	11.20	1.25
30.0	12.90	1.52
35.0	16.40	1.57
40.0	17.60	1.83
45.0	19.60	1.99
50.0	22.70	2.15
	$k_1^{app} = 4000\ M^{-1}\ s^{-1}$ [b]	
	$k_{-1}^{app} = 54\ M^{-1}\ s^{-1}$ [c]	
	$K^{app} = 72$	
	$k_2^{app} = 380\ M^{-1}\ s^{-1}$ [d]	

[a] [Methylcobalamin] = 5.0×10^{-5} M, at pH 4.50 using 0.10 M sodium acetate-acetic acid buffer. No additional ions were present. [b] Slope of k_Ψ^{fast} vs. $[Hg(OAc)_2]$. [c] Intercept of k_Ψ^{fast} vs. $[Hg(OAc)_2]$ divided by the stoichiometric acetate ion concentration (i.e., 1.8 s^{-1}/0.033 M acetate). [d] Slope of k_Ψ^{slow} vs. $[Hg(OAc)_2]$.

$[PdCl_4^{2-}] = 1.0 \times 10^{-3}$ M; $k_\Psi = 2.27 \times 10^{-3}\ s^{-1}$, $[PdCl_4^{2-}] = 2.0 \times 10^{-3}$ M; $k_\Psi = 3.96 \times 10^{-3}\ s^{-1}$, $[PdCl_4^{2-}] = 5.0 \times 10^{-3}$ M; $k_\Psi = 6.19 \times 10^{-3}\ s^{-1}$, $[PdCl_4^{2-}] = 8.0 \times 10^{-3}$ M; and $k_\Psi = 7.88\ s^{-1}$, $[PdCl_4^{2-}] = 1.0 \times 10^{-2}$ M) agreed well with that given in the literature ($9.0 \times 10^{-1}\ M^{-1}\ s^{-1}$ at 30 °C).[22]

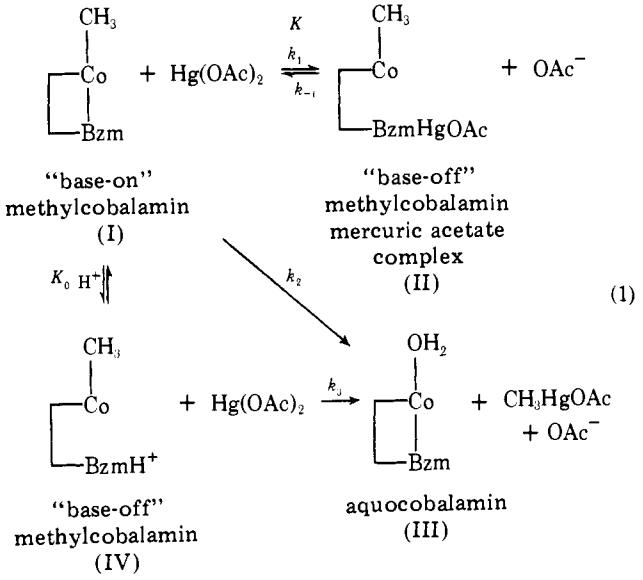
Mercuric acetate and potassium tetrachloropalladate were used as received (99.9% pure, Alfa Ventron). Reagent grade sodium dodecyl sulfate, SDS, was obtained from Biorad Laboratories and its purity was found to be satisfactory comparing its surface tension vs. concentration plot with authentically pure samples, generously provided by Kao Soap Co., Ltd., Japan. Purification of hexadecyltrimethylammonium bromide, CTABr,[8,11] and dodecylammonium propionate, DAP,[8] has been described. All other materials used were the best available grade. Water was doubly distilled from all glass equipment.

Stock solutions of methylcobalamin in water were 1.5×10^{-2} M. Mercuric acetate solutions were used within several hours after preparation in order to minimize precipitation of mercury as mercuric oxide. Data reported showed no evidence of HgO precipitation. Sodium dodecyl sulfate solutions were prepared with SDS from the two sources listed above and the same results obtained with each of them. Solutions were buffered using glycine or sodium acetate (glycine below pH 3.5 and acetate between 3.5 and 6.0). Measurements of pH were made on a Radiometer pH meter M-26.

Rates of methyl transfer from methylcobalamin to metal ion were followed spectrophotometrically in the thermostated cell compartment (25.0 ± 0.1 °C) of a Cary 118-C, Beckman Kintrac VII, and Durrum 110 stop-flow/temperature-jump systems at 350 nm. Experiments were carried out under pseudo-first-order conditions. Rates were followed up to completion and plots of log $(A_t - A_\infty)$ vs. time were linear up to ≥80% conversion in all cases. Second-order rate constants for methyl transfer were calculated from linear plots of the observed pseudo-first-order rate constants vs. metal ion concentration containing generally six points.

Results and Discussions

Reactions in Water. Addition of excess mercuric diacetate to methylcobalamin in buffered aqueous solutions at pH 4.5 results in the prompt development of a yellow color with an absorption maximum at 460 nm, followed by the slower reappearance of the red color, characterized by absorption maxima at 350, 497, and 523 nm (Figure 1). Similar spectral dependency for this process has been reported previously.[17] The spectrum due to the yellow intermediate has been assigned to the "base-off" methylcobalamin mercuric acetate adduct II and that due to the final product is identical with that of vitamin B_{12a}, aquocobalamin (III).[17,20] The following scheme appears to be consistent with the spectral and kinetic behavior of methylcobalamin with mercuric diacetate (eq 1). Under



(1)

appropriate conditions, pseudo-first-order rate constants have been determined for the formation of II, k_Ψ^{fast}, and of III, k_Ψ^{slow}, by stopped-flow spectrophotometry. Figure 2 shows typical oscilloscope traces and corresponding first-order rate plots. The data are in Table I. A good linear plot was obtained on treating the data for the complex formation according to the equation:

$$k_\Psi^{fast} = k_1^{app}[Hg(OAc)_2] + k_{-1}[OAc^-] \qquad (2)$$

Using eq 2, k_1^{app} was calculated to be $4000 \pm 200\ M^{-1}\ s^{-1}$ by computer treatment of the data. This value differs considerably from that reported previously ($k_1^{app} = 1.2\ M^{-1}\ s^{-1}$).[17] Taking the individual pseudo-first-order rate constants from Figure 3 in ref 17 and replotting them resulted, however, in a value of $3000\ M^{-1}\ s^{-1}$ for k_1^{app}. Thus, there is an apparent error in the calculation in ref 17. The value of $k_1^{app} = 4000\ M^{-1}\ s^{-1}$ is in accord with the expectation based on the rates of interaction of methylcobalamin with K_2PdCl_4.[22] Using the rate constant for the decomposition of II, $k_{-1}^{app} = 54\ M^{-1}\ s^{-1}$, in conjunction with k_1^{app} gave an equilibrium constant of $K = 72$ (Table I). This value is in good agreement with that determined previously ($K = 70$).[17] The determined rate constant for the slow reaction, $k_2^{app} = 380\ M^{-1}\ s^{-1}$, is also in accord with that reported previously ($k_2^{app} = 370\ M^{-1}\ s^{-1}$).[17]

Rate constants for the interaction of $Hg(OAc)_2$ with the "base-off" methylcobalamin, IV, were determined in 1.0 M

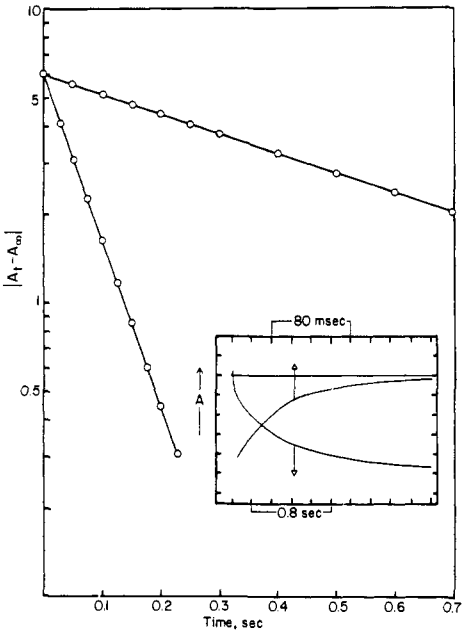


Figure 2. Insert shows the oscillographic trace of absorbance changes at 460 nm of 5.0×10^{-5} M methylcobalamin in the presence of 2.0×10^{-3} M $Hg(OAc)_2$ containing 0.10 M sodium acetate-acetic acid buffer at pH 4.5 and at 25.0 °C. The increase of absorbance corresponds to the formation of II, while the decrease of it is due to the formation of III. The figure shows the corresponding semilogarithmic plots for these absorbance changes.

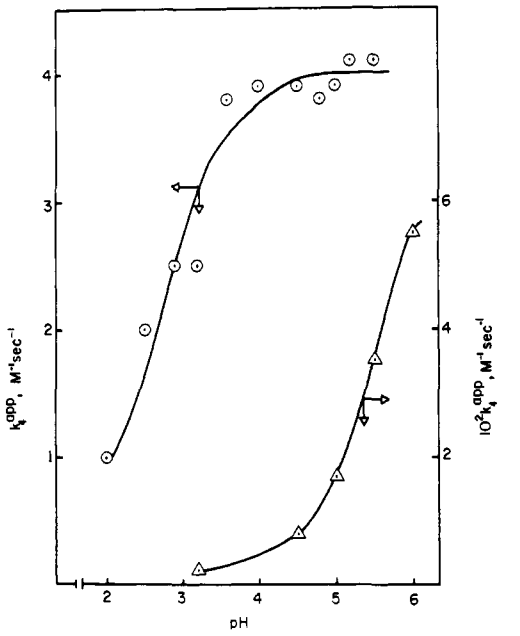


Figure 3. A plot of k_4^{app} vs. pH in aqueous 0.10 M sodium acetate buffer containing 0.10 M sodium chloride in the absence of (⊙) and in the presence of 0.10 M sodium dodecyl sulfate (△).

$HClO_4$ and the data are given in Table II. It is important to notice the strong dependence of the rate on the concentration of $Hg(OAc)_2$. In the concentration range of $(3.33\text{-}16.6) \times 10^{-3}$ M $Hg(OAc)_2$ the rate increases linearly with increasing concentration of the electrophile, giving a value of $k_3 = 9.07$ M^{-1} s^{-1} (Table II). As the concentration of $Hg(OAc)_2$ increases values for k_Ψ go through a maximum; then they decrease. At 1.0 M $Hg(OAc)_2$ the second-order rate constant, k_3, is 0.113 M^{-1} s^{-1}. This value corresponds well to those reported previously ($k_3 = 0.12$[17] and $k_3 = 0.064$ M^{-1} s^{-1} [18]). The reason for this substantial difference between k_3 values obtained at low and at high mercuric diacetate concentrations is likely to be due to the presence of different species as electrophiles at different concentrations. At low $Hg(OAc)_2$ concentrations in 1.0 M $HClO_4$ the mercury is present as free Hg^{2+}. At higher stoichiometric $Hg(OAc)_2$ concentrations mercury is no longer free. Rate constants for the interaction of methylcobalamin "base-off" (IV) with Hg^{2+} species (Hg^{2+}, $HgCl^+$, $HgCl_2$, $HgCl_3^-$, and $HgCl_4^{2-}$) have been determined by measuring rates of aquocobalamin formation in 1.00 M $HClO_4$ in the presence of added NaCl (Table III). Using stability constants of $K_{HgCl^+} = 10^{6.74}$, $K_{HgCl_2} = 10^{13.2}$, $K_{HgCl_3^-} = 10^{14.1}$, and $K_{HgCl_4^{2-}} = 10^{15.1}$ [23,24] allowed the calculation of the mole fractions of the different species ($f_{HgCl_n^{2-n}}$, where $n = 0, 1, 2, 3, 4$) present in each solution. These values allowed the calculation of the rate constant for the interaction of Hg^{2+} ($k_3^{Hg^{2+}}$), $HgCl^+$ ($k_3^{HgCl^+}$), $HgCl_2$ ($k_3^{HgCl_2}$), $HgCl_3^-$ ($k_3^{HgCl_3^-}$), and $HgCl_4^{2-}$ ($k_3^{HgCl_4^{2-}}$) with methylcobalamin base-off from the observed k_3 values assuming that

$$k_3^{obsd} = \sum_{n=0}^{n=4} k_3^{HgCl_n^{2-n}} \cdot f_{HgCl_n^{2-n}}$$ [25]

Values for the rate constants for the neutral and negatively charged mercury(II) species were found to be extremely small. Indeed a satisfactory fit between k_3^{obsd} and k_3^{calcd} values can be obtained by using $k_3^{Hg^{2+}} = 9.07$ and $k_3^{HgCl^+} = 1.85$ M^{-1} s^{-1} even in the range in which >99% of $HgCl_2$ is present in solution (Table III). The negligible reactivities of $HgCl_2$, $HgCl_3^-$, and $HgCl_4^{2-}$ as compared with Hg^{2+} and $HgCl^+$ are in accord with those observed for the relative reactivities of those species in the displacement of a manganese carbonyl group from a saturated carbon ($Hg^{2+}/HgCl^+/HgCl_2/HgCl_3^-/HgCl_4^{2-}$ = $41:2.5:1.7 \times 10^{-3}:2.0 \times 10^{-4}:10^{-5}$).[25]

Table II. Interaction of Methylcobalamin with $Hg(OAc)_2$ in 1.00 M $HClO_4$ at 25.0 °C[a]

$[Hg(OAc)_2]$, M	$10^2 k_\Psi$, s^{-1}
0.0033	3.80
0.0050	5.54
0.0066	6.93
0.0083	8.55
0.0100	10.50
0.0133	12.80
0.0166	13.90
0.0333	23.10
0.1000	46.00
1.0000[b]	11.30
$k_3 = 9.07$ M^{-1} s^{-1} [b]	
$k_3 = 0.113$ M^{-1} s^{-1} [c]	

[a] [Methylcobalamin] = 5.0×10^{-5} M. [b] Slope of k_Ψ vs. $[Hg(OAc)_2]$ in the range of 0.0033-0.0133 M $Hg(OAc)_2$. [c] Calculated from $k_\Psi/[Hg(OAc)_2]$ at 1.00 M $Hg(OAc)_2$.

At pH values where I predominates, formation of the "base-off" methylcobalamin mercuric acetate adduct II was not observable in the interaction of mercuric diacetate with methylcobalamin in the presence of excess sodium chloride. Furthermore, the pseudo-first-order rate constant for the formation of aquocobalamin decreased dramatically with increasing sodium chloride concentration from $k_\Psi = 125 \times 10^{-2}$ to $k_\Psi = 1.05 \times 10^{-2}$ s^{-1} (Table IV). Under this set of conditions the observed rate corresponds therefore to formation of aquocobalamin from methylcobalamin. Since this rate is independent of sodium chloride concentration in the range of $(1.6\text{-}16.6) \times 10^{-2}$ M salt (Table IV), the reaction in eq 1 simplifies to eq 3. This kinetic behavior is explicable in terms of the known stability constants for the different mercury(II) complexes. Using stability constants for the $HgCl_n^{2-n}$ species

Table III. Interaction of Methylcobalamin with Mercury(II) Species in 1.00 M $HClO_4$ in the Presence of Added NaCl at 25.0 °C[a]

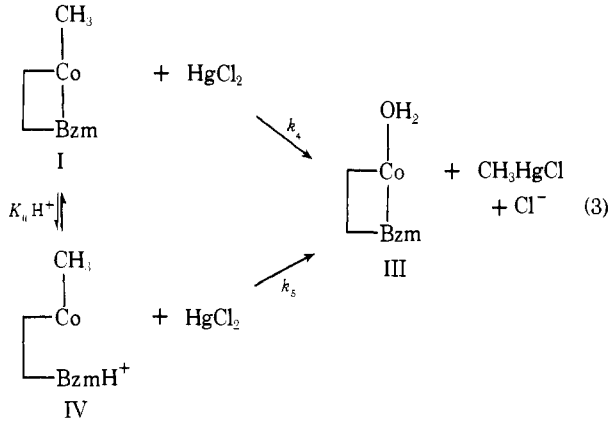
10^3[NaCl], M	$[Cl^-]_{free}$, M	% species present[b]			k_3^{obsd}, M^{-1} s^{-1}	k_3^{calcd},[c] M^{-1} s^{-1}
		Hg^{2+}	$HgCl^+$	$HgCl_2$		
0		100			9.00	9.07
1.00	1.82×10^{-8}	90.47	9.05	4.75×10^{-3}	8.55	8.37
3.00	5.60×10^{-8}	73.64	22.69	3.67	6.29	7.10
5.00	9.84×10^{-8}	59.02	31.92	9.06	5.86	5.94
7.00	1.48×10^{-7}	46.18	37.68	16.14	5.09	4.89
10.0	2.51×10^{-7}	29.55	40.83	29.62	3.22	3.41
11.0	2.98×10^{-7}	24.73	40.49	34.78	3.01	2.99
13.0	4.23×10^{-7}	16.21	37.72	46.07	2.10	2.17
15.0	6.39×10^{-7}	9.10	31.97	58.93	1.31	1.41
17.0	1.12×10^{-6}	3.72	22.83	63.45	0.50	0.51
19.0	3.30×10^{-6}	5.73×10^{-1}	9.47	90.00	0.17	0.18
20.5	5.76×10^{-5}	1.89×10^{-3}	5.98×10^{-1}	99.35	0.011	0.011
100.0	9.71×10^{-2}		1.41×10^{-4}	39.67	[d]	2.6×10^{-6}

[a] [Methylcobalamin] = 5.0×10^{-5} M; [$Hg(OAc)_2$] = 1.00×10^{-3} M. [b] $HgCl_3^-$ and $HgCl_4^{2-}$ are not given, since their reactivities are negligible. [c] Calculated from $k_3^{Hg^{2+}} = 9.07$ M^{-1} s^{-1}; $k_3^{HgCl^+} = 1.85$ M^{-1} s^{-1}; $k_3^{HgCl_2} = k_3^{HgCl_3^-} = k_3^{HgCl_4^{2-}} = 0$ using $k_3^{obsd} = \Sigma_{n=0}{}^{n=4} k_3^{HgCl_n^{2-n}} f_{HgCl_n^{2-n}}$. [d] <5% reaction in 14 days.

Table IV. Interaction of $Hg(OAc)_2$ in Water in the Presence of Added NaCl at pH 4.50 and 25.0 °C[a]

10^2[NaCl], M	$10^2 k_\Psi$, s^{-1}	10^2[NaCl], M	$10^2 k_\Psi$, s^{-1}
0	125	1.66	1.07, 1.08
0.166	24.8	3.30	1.05
0.333	16.9	6.60	1.07
0.500	9.24	10.0	1.05
0.660	3.85	13.3	1.05
1.00	1.73	16.6	1.05
1.33	1.28		

[a] [Methylcobalamin] = 5.0×10^{-5} M; [$Hg(OAc)_2$] = 2.5×10^{-3} M. Containing 0.10 M sodium acetate buffer.



(3)

(vide supra) and those for $K_{Hg(OAc)^+} = 10^{5.55}$, $K_{Hg(OAc)_2} = 10^{9.30}$, $K_{Hg(OAc)_3^-} = 10^{13.28}$, and $K_{Hg(OAc)_4^{2-}} = 10^{17.06}$,[23,24] and assuming no mixed complex formation, percentages of the different species present in the solution were calculated from mass balance equations. Thus, in 0.10 M sodium acetate–acetic acid buffer containing 0.10 M NaCl at pH ≤ 4.5 the only species present are the mercuric chlorides; there are 40% $HgCl_2$, 30% $HgCl_3^-$, and 30% $HgCl_4^{2-}$. At pH 4.8 in the same solution, in addition to mercuric chlorides, there is 0.28% $Hg(OAc)_4^{2-}$. It is seen that in solutions containing NaCl in excess of $Hg(OAc)_2$ the predominant species in solution are the different mercuric chloride complexes. For the sake of simplicity, the term $HgCl_2$ will be used to describe these complexes collectively in the ensuing discussion.

Table V summarizes the experimental data for the pH dependency of rate constants k_4 (in eq 3) for the formation of III. Decreasing the hydrogen ion concentration of the medium results in an increase in k_4 up to a maximum after which it levels off. Unfortunately a direct comparison of the pH rate profile with that reported[17] is not possible, since the earlier data was limited to pseudo-first-order rate constants utilizing 5.0×10^{-2} M $Hg(OAc)_2$ as the reagent in the absence of chloride ions. The kinetic data for the formation of aquocobalamin is described by:

$$k_4^{app} = k_4/(1 + ([H^+]/K_0)) + k_5/(1 + (K_0/[H^+])) \quad (4)$$

where k_4 and k_5 are pH independent rate constants for reactions described in eq 3. From the pH–rate profile (Figure 3) it is clear that the reactivity of the "base-off" methylcobalamin is very much smaller than that of the "base-on" methylcobalamin (i.e., $k_4 \gg k_5$). Neglecting the last term in eq 4 is, therefore, not unreasonable. The smaller reactivity of IV is expected in view of the Co–C bond strengthening subsequent to displacement of the 5,6-benzimidazole from the fifth coordination position. In the "base-off" methylcobalamin the electron density is removed from the cobalt atom, which renders it less susceptible to electrophilic attack. This type of kinetic trans effects has often been observed in ligand exchange reactions at cobalamins.[2] The assumption of a negligible value for k_5 is justified by the obtained agreement between the experimental pH–rate profile and that calculated using $k_4 = 4.0$ M^{-1} s^{-1}, $k_5 = 0$ and $pK_0 = 2.67$[2] in eq 3 (Figure 3). Hydrolysis of the Hg(II) species and the value of $pK_0 = 2.67$ precluded the dissection of the observed rate into reactivities of the individual mercury species in the pH range of 2–4.

Reactions in Aqueous Micelles. Addition of increasing amounts of sodium dodecyl sulfate (SDS) to aqueous solutions of methylcobalamin results in marked changes in the absorption spectra (Figure 4). Absorbances due to methylcobalamin "base-on" (I) at 520 nm decrease with the simultaneous appearance of a new absorbance centered at 470 nm, with an isosbestic point at 496 nm. The absorption spectra of the species formed in aqueous SDS corresponds to that observed for methylcobalamin in 1.0 M $HClO_4$. SDS promotes, therefore, the formation of the methylcobalamin "base-off" species (IV). The insert in the upper right hand side in Figure 4 illustrates the changes of absorbances at 465 nm as a function of added SDS. At surfactant concentrations $<1.0 \times 10^{-3}$ M there are no changes in the absorbances of methylcobalamin. Above this surfactant concentration, there is, however, a sigmoidal change reaching a plateau at 2.5×10^{-3} M surfactant con-

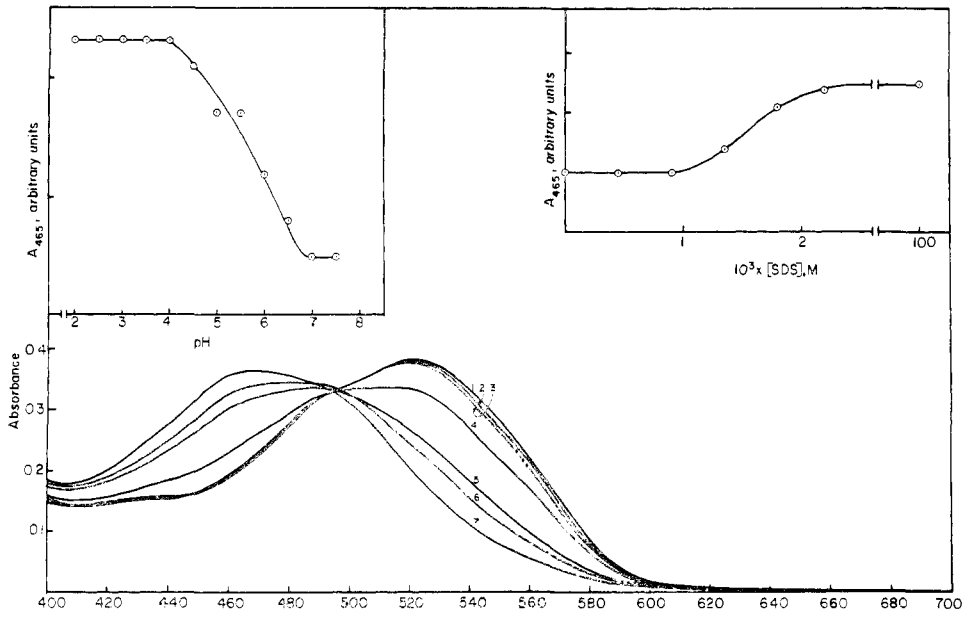


Figure 4. Absorption spectra of 5.0×10^{-5} M methylcobalamin at pH 5.0 and at 25.0 °C in 0.10 M sodium acetate buffer containing 0.10 M sodium chloride in the presence of 0 (1), 4.5×10^{-4} (2), 9.0×10^{-4} (3), 1.35×10^{-3} (4), 1.80×10^{-3} (5), 2.55×10^{-3} (6), and 0.10 M (7) sodium dodecyl sulfate. Upper left insert is a plot of the absorbance of methylcobalamin at 465 nm in the presence of 0.10 M SDS as a function of pH. Upper right insert is a plot of the absorbance of methylcobalamin at 465 nm as a function of sodium dodecyl sulfate concentration at pH 5.0.

centration. This behavior corresponds to the formation of SDS micelles which solubilize methylcobalamin. The critical micelle concentration of SDS in the presence of 0.1 M NaCl and methylcobalamin, determined from the data in Figure 4, 1.2×10^{-3} M, corresponds to those given in the literature in 0.10 M NaCl (1.5×10^{-3} [27] and 1.6×10^{-3} M[28]). The pH dependency of the absorbance due to I at 0.10 M SDS (upper left hand insert in Figure 4) allows the calculation of the pK_a for the formation of IV. A value of 5.67 is obtained which agrees well with that reported previously (5.73).[26] The corresponding pK_a value for the formation of "base-off" methylcobalamin in water is 2.63-2.72.[2,12,26] Micellar SDS affects dramatically, therefore, the equilibrium governed by K_0. The spectral data allows the calculation of the binding constant between the "base-off" methylcobalamin and micellar SDS, K_{IV}. Plotting the left hand side of eq 5 against the micelle concentration, M

$$\log \frac{A_0 - A}{A - A_\infty} = \log K_{IV} + n_H \log [M] \qquad (5)$$

(where A_0, A_∞, and A are absorbances of the free methylcobalamin, that completely in the form of the complex, and that in any intermediate forms between, and [M] = stoichiometric surfactant concentration (cmc)) resulted in a good straight line from the intercept of which K_{IV} was calculated to be 5000 M^{-1}. Apparently, micellar SDS binds fairly strongly to IV.

Rate constants for the interaction of mercuric dichloride with methylcobalamin, k_4^{app} values, decrease with increasing SDS concentration to a minimum value, after which they level off (Table VI and Figure 5). At the highest concentration of SDS the rate constant is some 230 times smaller than that in pure water. Effects of aqueous micelles on reaction rates have been discussed in terms of partitioning of the reactants between the micellar pseudophase and bulk water and in terms of differential reaction rates in these two phases.[8] Berezin and his co-workers have derived equations for the quantitative treatment of micellar catalysis and inhibition.[29] Equation 6 accounts for the observed rate constant for the interaction of mercuric dichloride with methylcobalamin in micellar SDS:

$$k_4^{app} = \frac{k_4^M(P_{IV} + P_I)P_{HgCl_2}MV + k_4^W(1 - MV)}{[1 + (K_{IV} + K_I)M]\,(1 + K_{HgCl_2}M)} \qquad (6)$$

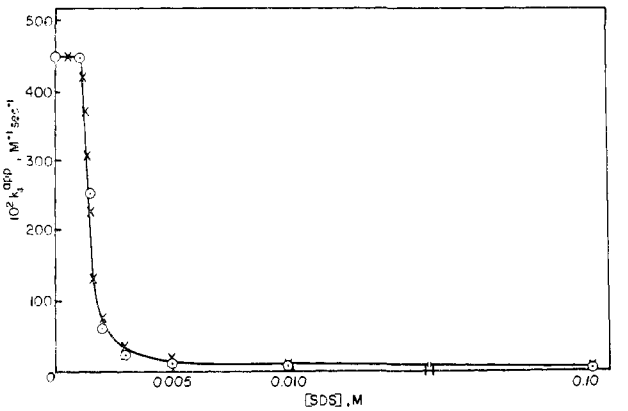


Figure 5. Dependence of k_4^{app} on the concentration of SDS at pH 5.00. Experimental points are indicated by ⊙ and those calculated by the use of eq 7 are indicated by X.

where k_4^M and k_4^W are rate constants in the micellar and aqueous bulk phases, P_{IV}, P_I, and P_{HgCl_2} are partition coefficients of IV, I, and $HgCl_2$ between the micellar phase and water, K_{IV}, K_I, and K_{HgCl_2} are binding constants of IV, I, and $HgCl_2$ to the micelle, M is the concentration of the micelle, and V is the molar volume of the surfactant. Equation 6 may be simplified by making three assumptions. Firstly, the rate in the micellar phase is very much smaller than that in water and can therefore be neglected, i.e., $k_4^M \simeq 0$. This is not an unreasonable assumption, since the much less reactive IV predominates over I in micellar SDS. Secondly, using previously reported data,[26] in the range of $(2\text{-}4) \times 10^{-3}$ M SDS (the range of surfactant concentration where the most pronounced rate inhibition is observed, Table VI) values for K_{IV} and K_I are calculated to be 1000-10 000 and 1-10 M^{-1}, respectively. Since K_I is at least 1000-fold smaller than K_{IV}, it is neglected. Finally, at relatively low surfactant concentrations, the micellar volume is negligible compared to the volume of water, i.e., $(1 - MV) = 1$. The simplified equation:

$$k_4^{app} = k_4^W/[(1 + K_{IV}M)(1 + K_{HgCl_2}M)] \qquad (7)$$

Table V. Interaction of Methylcobalamin with $HgCl_2$ in Water at 25.0 °C[a]

pH[b]	$10^4[HgCl_2]$, M	10^3k_Ψ, s^{-1}	k_4^{app}, M^{-1} s^{-1}
2.00	5.00	0.45	1.00
	10.0	0.92	
	16.7	1.61	
	25.0	2.52	
	36.7	4.01	
	50.0	5.68	
2.50	5.0	0.90	2.00
	10.0	1.94	
	16.7	3.01	
	25.0	4.71	
	36.7	7.20	
	50.0	10.30	
2.90	10.0	2.18	2.50
	16.7	3.91	
	33.3	7.87	
	50.0	12.10	
3.20	5.0	1.16	2.50
	10.0	2.37	
	16.7	4.15	
	25.0	6.03	
	36.7	8.45	
	50.0	10.70	
3.60	10.0	3.20	3.80
	16.7	5.72	
	33.3	11.90	
	50.0	18.20	
4.00	5.0	1.60	3.90
	10.0	3.85	
	16.7	6.79	
	25.0	10.20	
	36.7	14.70	
	50.0	21.00	
4.50	5.0	2.31	3.90
	10.0	3.85	
	20.0	7.88	
	25.0	9.90	
	30.0	11.60	
	35.0	13.30	
	40.0	14.70	
	50.0	18.70	
4.80	10.0	3.20	3.80
	16.7	6.12	
	33.3	12.20	
	50.0	18.70	
5.00	5.0	1.63	3.20
	10.0	3.43	
	16.7	5.78	
	25.0	7.97	
	36.7	13.10	
	50.0	18.70	
5.20	10.0	3.10	4.10
	16.7	6.04	
	33.3	12.60	
	50.0	19.80	
5.50	10.0	3.52	4.10
	16.7	5.92	
	25.0	9.00	
	36.7	14.40	
	50.0	21.70	

[a] [Methylcobalamin] = 5.0×10^{-5} M. All solutions contain ca. 0.10 M NaCl. [b] Using 0.10 M glycine buffer at pH 2.00, 2.50, 2.90, and 3.20; and 0.10 M sodium acetate at pH 3.60, 4.00, 4.80, 5.00, 5.20, and 5.50.

may be rearranged to equation:

$$(k_4^W/k_4^{app} - 1)/M = (K_{1V} + K_{HgCl_2}) + K_{1V}K_{HgCl_2}M \quad (8)$$

Plotting the data according to eq 8 resulted in a good straight line which allowed the calculation of K_{1V} and K_{HgCl_2} from the slopes and intercepts to be 4950 and 55 M^{-1}, respectively. The excellent agreement between the kinetically and spectroscopically calculated binding constant between the "base-off" methylcobalamin and SDS lends credence to the various assumptions made in the derivation of eq 7. The binding constant for $HgCl_2$ is small and reflects the binding of all mercuric species (vide supra). The assumptions made in deriving eq 7 are further substantiated by the satisfactory agreement between the experimentally obtained k_4^{app} values and those calculated by eq 7 (Figure 5).

Table VI. Interaction of Methylcobalamin with $HgCl_2$ in Aqueous Sodium Dodecyl Sulfate (SDS) at pH 5.00 and at 25.0 °C[a]

10^3[SDS], M	$10^3[Hg(Cl)_2]$, M	10^3k_Ψ, s^{-1}	k_4^{app}, M^{-1} s^{-1}
1.00	1.00	3.42	3.90
	2.00	7.53	
	3.33	12.30	
	3.67	13.50	
	4.50	17.80	
	5.00	18.90	
1.50	1.00	3.01	2.70
	2.00	5.89	
	3.33	9.52	
	3.67	10.10	
	4.50	12.40	
	5.00	13.60	
2.00	1.00	0.89	0.65
	2.00	1.74	
	3.33	2.38	
	3.67	2.88	
	4.50	3.30	
	5.00	3.50	
3.00	1.00	0.39	0.30
	2.00	0.75	
	3.33	1.11	
	4.50	1.46	
	5.00	1.53	
5.00	1.00	0.20	0.15
	2.00	0.43	
	3.33	0.59	
	3.67	0.64	
	4.50	0.77	
	5.00	0.83	
10.00	1.00	0.14	0.061
	2.00	0.17	
	3.33	0.26	
	3.67	0.28	
	4.50	0.33	
	5.00	0.35	
100.00	1.00	0.023	0.017
	2.50	0.048	
	5.00	0.090	
	10.00	0.13	
	30.00	0.34	
	50.00	1.11	

[a] [Methylcobalamin] = 5.0×10^{-5} M; containing 0.10 M sodium acetate buffer and 0.10 M NaCl.

In 0.10 M SDS k_4^{app} values increase exponentially with increasing pH values (Table VII). Precipitation of HgO precluded the observation rate constants at pH >6.0. It is evident that the pH–rate profile in 0.10 M SDS shifted to 3 pH units higher than that observed in water (Figure 3). This is a consequence of the alteration of the protonation equilibrium, governed by K_0. The pH dependency of k_4^{app} was treated analogously to that in water. Substituting $pK_0 = 5.73$ into eq 4, the pH independent rate constant for the attack of $HgCl_2$ on IV, k_4, in 0.10 M SDS was calculated to be 0.11 M^{-1} s^{-1}. This value is a factor of 36 times smaller than that observed in water and can be attributed to differential rate of electro-

Table VII. Interaction of Methylcobalamin with $HgCl_2$ in Aqueous 0.10 M Sodium Dodecyl Sulfate (SDS) at 25.0 °C[a]

pH[b]	$10^3[HgCl_2]$, M	10^3k_Ψ, s^{-1}	$10^3k_4^{app}$, M^{-1} s^{-1}
4.50[c]	1.00	0.135	130
	2.00	0.285	
	3.00	0.385	
	4.00	0.462	
	5.00	0.559	
	6.00	0.831	
	7.00	0.916	
4.50	1.00	0.008	8.10
	2.00	0.015	
	3.33	0.026	
	3.67	0.029	
	4.50	0.038	
	5.00	0.039	
5.00	1.00	0.023	17.4
	2.50	0.048	
	5.00	0.090	
	10.0	0.13	
	30.0	0.34	
	50.0	1.11	
5.50	10.0	0.28	35.0
	16.7	0.47	
	25.0	0.75	
	33.3	1.16	
	50.0	2.06	
6.00	10.0	0.39	55.1
	16.7	0.75	
	25.0	1.22	
	33.3	1.65	
	50.0	2.63	

[a] [Methylcobalamin] = 5.0×10^{-5} M. [b] 0.10 M sodium acetate buffer, containing 0.10 M NaCl, unless specified otherwise. [c] 0.10 M sodium acetate-acetic acid buffer, containing no sodium chloride, the second rate constant therefore k_2^{app} (reaction 1) and the reagent is $Hg(OAc)_2$ rather than $HgCl_2$.

philic attack of $HgCl_2$ on IV in micellar and aqueous environment.

Inhibition of the reaction, governed by k_4, by micellar SDS has been demonstrated to originate in the selective uptake of IV in the micelle, where the reaction is slower than that in water. The solubilization is likely to be similar to that proposed for aquocobalamin.[10] The benzimidazole moiety is envisaged to be buried in the micelle and is assumed to be in the environment of the first few CH_2 groups next to the surfactant headgroup, while the methyl group in the sixth coordination is in contact with and is hydrated by water in the Gouy-Chapman electrical double layer. The effective hydrogen ion concentration in the negatively charged micellar surface and below it is likely to be considerably higher than that in bulk water. This increase in the effective hydrogen ion concentration results in the significant increase in the dissociation constant for the formation of IV. Over and above this solubilization effects there is a "genuine" 36-fold micellar rate retardation.

Table VII also contains data for the effect of 0.10 M SDS on the interaction of $Hg(OAc)_2$ with methylcobalamin, reaction 1, at pH 4.50. The obtained rate constant, $k_2^{app} = 0.13$ M^{-1} s^{-1}, should be compared to that in water, $k_2^{app} = 380$ M^{-1} s^{-1} (Table I). The micellar rate retardation is, once again, a composite effect of that on K_0 and that on k_2. Using a value of $pK_0 = 5.73$ in 0.10 M SDS, the pH independent rate constant in 0.10 M SDS is calculated to be $k_2 = 2.33$ M^{-1} s^{-1}. A comparison of this value with that in water ($k_2 = 380$ M^{-1} s^{-1}, Table I) indicates that micellar inhibition due to differential rates in the two environments is 160-fold. Apparently, reaction

Table VIII. Interaction of Methylcobalamin with $Hg(OAc)_2$ in Water in the Presence of NaBr at pH 5.00 and at 25.0 °C[a]

[NaBr], M	$10^3[Hg(OAc)_2]$, M	10^3k_Ψ, s^{-1}	k_{4a}^{app}, M^{-1} s^{-1} [c]
0[b]			0.167
0.10	1.00	0.16	
	2.00	0.31	
	3.33	0.55	
	3.67	0.61	
	4.50	0.78	
	5.00	0.92	
2.00	5.00	0.032	0.0064

[a] [Methylcobalamin] = 5.0×10^{-5} M, containing 0.10 M sodium acetate-acetic acid buffer. [b] See Table I. [c] k_{4a} refers to eq 3, but the main species in solution are the mercuric bromide rather than the mercuric chloride complexes (see Results and Discussion).

IV with OAc^- is retarded by micellar SDS to a greater extent than that with Cl^-.

The reaction of mercuric diacetate with methylcobalamin is completely supressed by the addition of hexadecyltrimethylammonium bromide, CTABr. No changes in the absorption spectra of 5.0×10^{-5} M methylcobalamin in buffered (0.10 M sodium acetate-acetic acid, pH 5.0) aqueous 0.010 or 0.10 M CTABr solutions were observed for 48 h at 25.0 °C subsequent to the addition of 1.0×10^{-3} M $Hg(OAc)_2$. In order to understand the mechanism for this micellar rate retardation, rate constants for aquocobalamin formation were determined as a function of increasing sodium bromide concentrations. At 0.10 M NaBr concentration, increasing the concentration of $Hg(OAc)_2$ results in a linear increase of the pseudo-first-order rate constant for the formation of aquocobalamin (Table VIII). The second-order rate constant decreases exponentially with increasing sodium bromide concentrations. Using the reported stability constants of $K_{HgBr^+} = 10^9$, $K_{HgBr_2} = 10^{17.1}$, $K_{HgBr_3^-} = 10^{19.4}$, and $K_{HgBr_4^{2-}} = 10^{21}$,[23] and assuming no mixed complex formation percentages of the different species in 0.10 M NaBr and 5.0×10^{-3} M $Hg(OAc)_2$, solutions were calculated to be $HgBr_2$ = 1.0%, $HgBr_3^-$ = 20%, and $HgBr_4^{2-}$ = 79%. Similarly, in 2.0 M NaBr and 5.0×10^{-3} M $Hg(OAc)_2$ there are 0.6% $HgBr_3^-$ and 99.4% $HgBr_4^{2-}$. Thus, the predominant species in solutions are the mercuric bromides. Since the effective counterion concentration in the Stern layer of micelles is approximately 3.0 M[30] the observed complete inhibition cannot be entirely attributed to the reaction of $HgBr_4^{2-}$. A plausible explanation for this complete rate annihilation is that while methylcobalamin is not solubilized, $HgBr_4^{2-}$ effectively partitions onto the micellar CTABr.[26] Thus the reactive partners have a diminished probability of encountering each other.

Reactions in Reversed Micelles. Methylcobalamin is completely insoluble in benzene. Aqueous solutions of it are readily solubilized, however, by dodecylammonium propionate, DAP. By analogy with vitamin B_{12} the corrinoid is localized in the water pool, which is separated from benzene by several hundred surfactant molecules.[11] The absorption spectra of 5.0×10^{-5} M methylcobalamin entrapped in 1.0 M water by 0.20 M DAP in benzene corresponds entirely to that of the "base-on" methylcobalamin, I. The effective microenvironment of the corrinoid in this large water pool is estimated to be only slightly less polar than that provided by bulk water.[11]

Addition of mercuric diacetate to the surfactant solubilized methylcobalamin in benzene results in the formation of hydroxocobalamin. Since the pK_a for the formation of hydroxocobalamin in 0.2 M DAP solubilized water pools is 7.6 ± 0.4,[11] the effective pH of this environment is in the vicinity of 8. It is interesting to observe that in bulk water precipitation

Table IX. Interaction of Methylcobalamin with $Hg(OAc)_2$ in Benzene in the Presence of 0.20 M Dodecylammonium Propionate at 25.0 °C[a]

$10^4[Hg(OAc)_2]$, M	$10^5 k_\psi$, s^{-1}
3.33	1.97
6.66	2.44
10.0	3.17
13.3	3.72
16.6	4.65
20.0	5.60
23.3	6.01
$10^2 k_1 = 2.13\ M^{-1}\ s^{-1}$	

[a] [Methylcobalamin] = 5.0×10^{-5} M, containing 1.00 M water.

of mercuric oxide occurs at pH 8 in the mercuric diacetate concentration range of $(3\text{–}23) \times 10^{-4}$ M. Apparently reversed micelles shift the equilibrium of hydrolysis of mercuric diacetate. The rate constants for the formation of hydroxocobalamin in the surfactant solubilized water pool in benzene is considerably smaller than that for the formation of aquocobalamin in water (Tables I and IX). The observed first-order rate constants increase linearly with increasing $Hg(OAc)_2$ concentration (Table IX). Although there is no information on the stability constants of mercury(II) propionate complexes, their values are likely to correspond to those of the mercury(II) acetate complexes. Under these experimental conditions, the rate constant for methylcobalamin decomposition in the DAP solubilized water pool is approximately 18 000-fold smaller than that in bulk water. This large rate inhibition is likely to be the consequence of tying up the mercury to the surfactant head groups in the reversed micellar cavity.

Conclusion

Rate constants for methyl transfer from methylcobalamin to mercury(II) in water depends strongly on the nature of the anion. The reactivities are in the order acetate > chloride > bromide. This order is the opposite to that observed for the stabilities of the corresponding mercury(II) complexes and supports, therefore, a mechanism in which predominantly the cationic Hg(II) species act as the attacking electrophile. This postulate is substantiated by the observed dependence of the rate on $Hg(OAc)_2$ concentration in 1.0 M $HClO_4$.

The most significant fact emerging from the observed micellar inhibition on the rate of methyl transfer is the role negatively charged aggregates play in shifting the pH–rate profile for the formation of III, and the pK_a value for benzimidazole displacement to 3 higher pH units. The pK_a for this process in micellar SDS, 5.67, is in the range of physiological pH. Enzyme mediated microscopic acidity changes are likely to result, therefore, in substantial changes of methyl transfer rates.

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