Effects of Surfactants on Ligand Exchange Reactions in Vitamin B_{12a} in Water and in Benzene. Influence of Aqueous Micelles and of Solvent Restrictions

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Abstract: Rate constants for the formation, k_1^{app}, and decomposition, k_{-1}^{app}, of glycine, imidazole, and sodium azide adducts of vitamin B_{12a} have been determined in aqueous micellar hexadecyltrimethylammonium bromide and sodium dodecyl sulfate as well as in dodecylammonium propionate and sodium di(2-ethylhexyl)sulfosuccinate solubilized water in benzene. Effects in aqueous micelles are relatively small and are rationalized on electrostatic considerations. Using the obtained linear correlations between the absorption maxima of vitamin B_{12a}, and glycine solubilities *vs.* solvent polarity parameters $E_T(30)$, the effective environments of the reactants in surfactant solubilized water in benzene were determined to depend on the concentrations of water and of surfactants. Vitamin B_{12a} molecule is effectively shielded from the apolar solvent by some 300 surfactant molecules. Values for k_1^{app} and k_{-1}^{app} for the interaction of vitamin B_{12a} with glycine in the polar cavities of surfactants in benzene are significantly greater than those in bulk water. At constant concentration of solubilized water k_1^{app} and k_{-1}^{app} increase with increasing DAP concentration up to a maximum after which they decrease logarithmically. The maxima of rate enhancements increase with decreasing water concentrations. At relatively high water and surfactant concentrations the rate and equilibrium constants for the formation of the imidazole complex of vitamin B_{12a} in benzene are essentially identical with those in bulk water. In benzene in the presence of 0.02 *M* DAP and 8.9×10^{-2} *M* water the observed rate constant for the equilibrium attainment of the imidazole vitamin B_{12a} adduct decreases logarithmically with increasing concentration of imidazole. Substantial rate enhancement, with respect to water, is obtained at the lowest imidazole concentration. Values of k_1^{app} and k_{-1}^{app} for the interaction of sodium azide with vitamin B_{12a} in benzene in the presence of DAP are markedly smaller than those in water. Significance of these results is discussed.

Rate constants for the formation, k_1, and dissociation, k_{-1}, of several cobalamins formed from vitamin B_{12a}, Bzm-Co-OH_2, have been recently determined in aqueous solutions (eq 1).[1-7] Since the

$$\text{Bzm-Co-OH}_2 + \text{Y} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{Bzm-Co-Y} + \text{H}_2\text{O} \qquad (1)$$

microenvironment of vitamin B_{12a} and its analogs *in vivo* is likely to be different from that in water, the obtained kinetic parameters incompletely represent the processes occurring in living systems. Polar cavities of surfactant aggregates in apolar solvents, reversed micelles, provide a unique reaction media which may well approximate the active sites of enzymes.[8] Indeed, highly specific and substantial rate enhancements have been observed for reactions in reversed micellar systems.[9] The present paper reports that surfactant aggregates in benzene are capable of solubilizing such a large molecule as vitamin B_{12a} and that these systems provide an eminently suitable media for the investigation of secondary valence force interactions on cobalamin reactivity. For the sake of comparison we have also examined the effects of aqueous micelles on selected ligand exchange reactions of vitamin B_{12a}.

Experimental Section

Best available grade of vitamin B_{12}, cyanocobalamin (Sigma), and vitamin B_{12a}, aquocobalamin (Merck), was used as received. Their purity was established by spectrophotometry and by obtaining rate constants for ligand exchange reactions in water identical with those reported previously (*vide infra*). Preparation and purification of dodecylammonium propionate (DAP), sodium di(2-ethylhexyl)-sulfosuccinate (Aerosol-OT), hexadecyltrimethylammonium bromide (CTAB), and sodium dodecyl sulfate (NaLS) have been described.[9,10] Aqueous solutions were prepared in doubly distilled water. The pH was determined by a Radiometer PHM-26 instrument. Reagent grade benzene was distilled from sodium and stored over Linde 5A molecular sieve. Stock solutions of vitamin B_{12a} (usually $2\text{-}8 \times 10^{-3}$ *M*) were made up in water and stored in the refrigerator. Individual solutions for spectral and kinetic determinations were prepared by injecting appropriate volumes of the aqueous vitamin B_{12a} stock solutions to benzene solutions of the surfactants. Final concentrations of vitamin B_{12a} ranged between 5×10^{-7} and 500×10^{-7} *M*. Water concentrations, 0.01–1.1 *M*, were carefully controlled and monitored by gas–liquid partition chromatography using a porapak Q column. All other chemicals used were the best available reagent grade.

The glycine complex of vitamin B_{12a} was isolated by stirring equal volumes of aqueous solutions of vitamin B_{12a} (5×10^{-3} *M*) with glycine (0.30 *M*) for 3 hr at room temperature. A small volume (1.0 ml) of this solution was added to a 100-ml benzene solution of 0.10 *M* Igepal CO-530. The glycine complex of B_{12a} precipitated in this system while the excess glycine was solubilized. The precipitate was filtered, washed with acetone, and dried *in vacuo* over P_2O_5. The absorption spectra of the isolated glycine complex agreed in all respects with that formed *in situ* from vitamin B_{12a} and glycine both in water and in benzene in the presence of DAP. Agreement between the rate constants for the decay of the isolated glycine complex of vitamin B_{12a} and that obtained in its *in situ*

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Table I. Absorption Spectra of Vitamin B_{12} in Different Solvents

Solvent system[a]	α band		β band			γ band					
	λ_{max}[b]	ϵ[b]	λ_{max}	ϵ	λ_{max}	ϵ	λ_{max}	ϵ	λ_{max}	ϵ	λ_{max}	ϵ
Water, pH 6.9 [$E_T(30) = 63.1$]	550.4 (552)	7.9×10^3	519.5 (520)	7.1×10^3	408.7	3.3×10^3	361.1 (360.5)	2.6×10^4	322.3 (322)	7.4×10^2	305.5 (305)	8.7×10^3	278.2 (277)	1.5×10^4
Ethylene glycol [$E_T(30) = 56.3$]	549.2	8.5×10^3	518.0	7.4×10^3			362.5	2.7×10^4	322.5	8.3×10^3	306.0	9.5×10^3	278.6	1.6×10^4
Methanol [$E_T(30) = 55.5$]	548.6	8.4×10^3	517.3	7.3×10^3			360.9	3.0×10^4	322.4	7.9×10^3	305.9	9.0×10^3	278.5	1.6×10^4
Ethanol [$E_T(30) = 51.9$]	549.2		517.9						322.5		306.1		278.5	
1-Butanol [$E_T(30) = 50.2$]	549.2		517.8				361.2		322.8		306.1		278.6	
2-Propanol [$E_T(30) = 50.7$]	549.1		517.5				361.1		322.5		304.2		278.7	
DMSO [$E_T(30) = 45.0$]	547.6 (547)	7.7×10^3	516.9 (516)	6.7×10^3	396.0	3.2×10^3	361.0 (363)	2.3×10^4	323.4 (325)	7.7×10^3	308.0 (309)	8.1×10^3	279.9	1.0×10^4
Pyridine E_T[(30 = (40.2]	547.0 (547)		516.0 (516)											
5.0×10^{-2} *M* CTAB in H_2O	550.4	4.0×10^3	519.6	3.6×10^3	407.7	1.8×10^3	361.3	1.3×10^4	322.4	3.7×10^3	305.4	4.4×10^3	278.2	7.3×10^3
5.0×10^{-1} *M* NaLS in H_2O	549.6	1.0×10^4	518.8	8.9×10^4			361.0	3.4×10^4	323.2	1.8×10^4	306.5	2.0×10^4	278.5	2.7×10^4
0.20 *M* DAP, 0.033 *M* H_2O in C_6H_6	546.8	8.2×10^3	515.5	7.2×10^3			360.5	2.8×10^4						
0.20 *M* DAP, 0.074 *M* H_2O in C_6H_6	547.8	9.7×10^3	516.6	8.7×10^3			360.9	2.7×10^4						
0.20 *M* DAP, 0.13 *M* H_2O in C_6H_6	549.2	9.1×10^3	518.3	8.3×10^3			361.5	2.6×10^4						
0.20 *M* DAP, 0.30 *M* H_2O in C_6H_6	550.0	8.4×10^3	518.0	7.6×10^3			361.1	2.7×10^4						
0.20 *M* DAP, 1.1 *M* H_2O in C_6H_6	550.4	7.4×10^3	519.2	6.5×10^3	407.6		361.2	3.4×10^4						
0.02 *M* DAP, 0.01 *M* H_2O in C_6H_6	550.0		518.3											
0.02 *M* DAP, 0.05 *M* H_2O in C_6H_6	550.0		519.2											
0.02 *M* DAP, 0.10 *M* H_2O in C_6H_6	551.0		519.3											
0.02 *M* DAP, 0.15 *M* H_2O in C_6H_6	552.8		519.5											
0.02 *M* DAP, 0.20 *M* H_2O in C_6H_6	554.0		519.2											

[a] Values in parentheses are taken from J. A. Hill, J. M. Pratt, and R. J. P. Williams, *J. Chem. Soc.*, 5149 (1964). [b] λ_{max} in nm; ϵ in M^{-1} cm^{-1}.

generation (*vide infra*) also substantiates the proposed structure of the isolated species.

Spectrophotometric determinations were carried out using a Cary 118-C spectrophotometer whose cell compartment was thermostated at 24.8 ± 0.1°. Initially, the complete spectral range was recorded, generally on the 0–2.0-*A* scale at a speed of 10 nm/in. and 0.2 nm/sec. For absorption maxima determinations the top of the offset peaks were rerun three times at 0.005 nm/sec and 2 nm/in. on the 0–0.02-*A* scale. The accuracy of the absorption maxima is ±0.05 nm. Kinetic data were obtained on a Cary 118-C and on a Beckman Kintrac VII spectrometer. The faster runs (in the aqueous solutions only) were followed by a Durrum Model 110 stopped-flow spectrometer. Temperatures for the kinetic runs were maintained at 24.8 ± 0.1° by water circulation.

Solubilities of vitamin B_{12a} were determined by measuring concentrations of saturated solutions spectrophotometrically. Excess solid vitamin B_{12a} was added to a series of benzene solutions containing different amounts of DAP. These solutions were vigorously shaken, allowed to stand overnight at 25.0°, and centrifuged. Diluted aliquot parts were used to determine absorbances at 350 nm using appropriate blank solutions. Concentrations of the saturated solutions were then calculated using $\epsilon = 2.0 \times 10^4$ M^{-1} cm^{-1}. Solubilities of glycine were determined by forming the *n*-trifluoroacetyl *n*-butyl ester of glycine[11] in diluted portions of the centrifuged aliquots of saturated benzene solutions of glycine containing different amounts of DAP. Concentrations of the *n*-trifluoroacetyl *n*-butyl ester derivatives of glycine were quantitatively determined by gas–liquid partition chromatography using the ester derivative of phenylalanine as an internal standard. Details of this analysis followed that described in the literature[11] and good reproducibilities were obtained on glycine solutions of known concentrations.

The dissociation constants of vitamin B_{12a} were determined spectrophotometrically. The pH of a stock solution of 5.0×10^{-3} *M* vitamin B_{12a} in 5.0×10^{-2} *M* CH_3COONa and 5.0×10^{-2} *M* Na_2HPO_4 aqueous buffer was adjusted by NaOH and HCl. Small portions (50 μl) of the aqueous buffered vitamin B_{12a} solutions were injected into aqueous buffers or benzene solutions containing 0.1 *M* DAP. The final B_{12a} concentration in these solutions was 5.0×10^{-5} *M*. The dissociation constants of imidazole and sodium azide in aqueous micellar CTAB and NaLS were determined by titration using 0.1 *N* HCl.

Results

Parameters for the absorption spectra of vitamin B_{12} in different environments are given in Table I. The absorption maxima of the α and β bands are markedly solvent dependent. A good correlation is obtained, in fact, between these absorption maxima and the solvent polarity parameter $E_T(30)$[12] (Figure 1). Such correlation allows the estimation of the microscopic polarities of the environment in which vitamin B_{12} is solubilized in different surfactant solutions. The appropriate data are also included in Table I and absorbance maxima of selective systems are indicated in Figure 1.

Vitamin B_{12a} is highly soluble in water. It is, however, completely insoluble in benzene. With our detection methods (absorption spectroscopy using 10.0-cm cells) we would have been able to detect $\leq 10^{-8}$ *M* vitamin B_{12a} in saturated benzene solutions. This value can, therefore, be considered as the upper limit of solubility in benzene. Addition of DAP solubilizes vitamin B_{12a} in benzene (Table II and Figure 2). It is interesting to note that the solubility of vitamin B_{12a} increases linearly with increasing DAP concentrations up to a point after which the solubility decreases. This point of break depends on the amount of water present in the benzene–DAP system. At higher water concentration the break occurs at higher DAP concentrations (Table II). Evidently water is also required to solubilize vitamin

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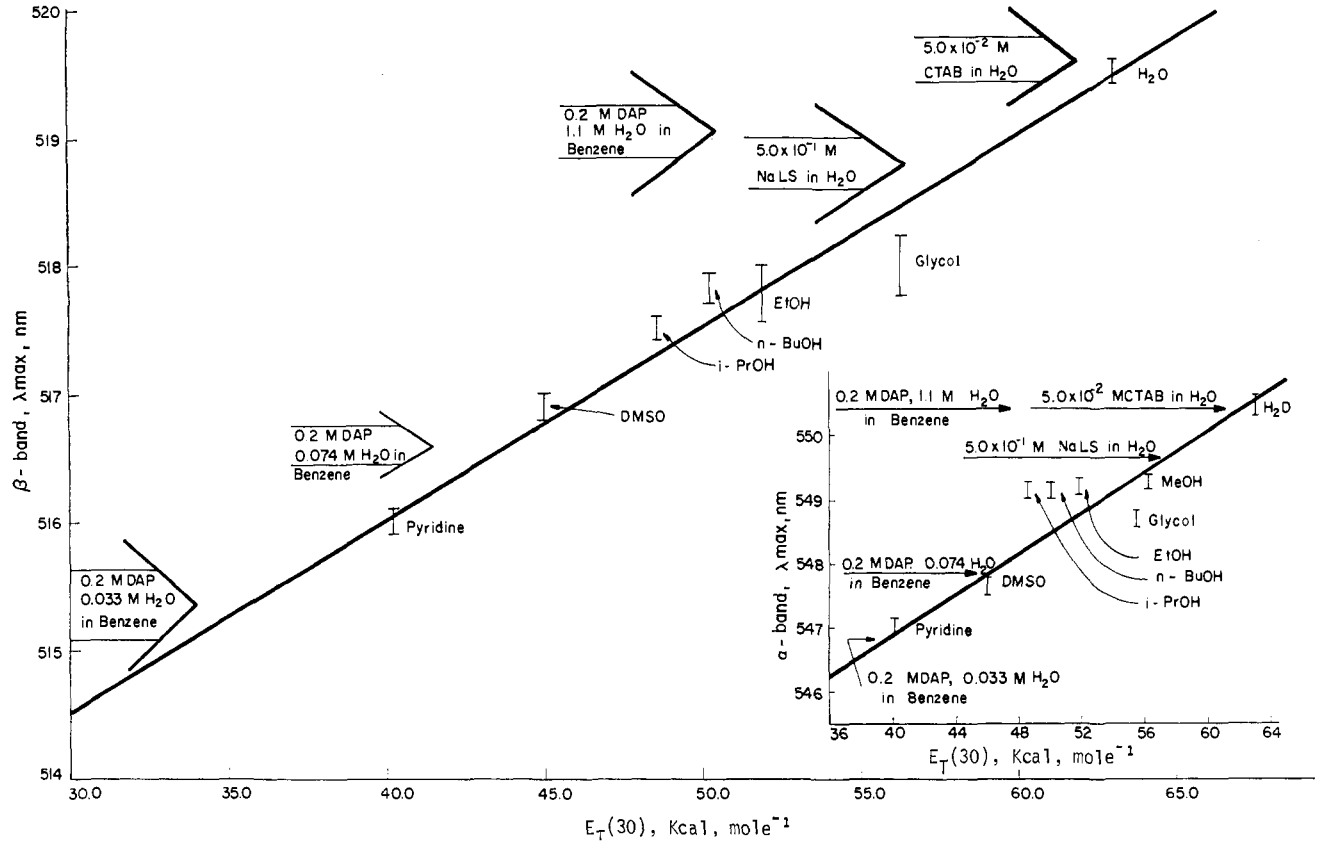

Figure 1. Correlation between the absorption maxima of the α and β bands of vitamin B_{12} and solvent polarity parameter $E_T(30)$.

Table II. Solubility of Vitamin B_{12a} in Benzene in the Presence of DAP at 25.0°[a]

10^2[DAP], M	Solubility, $M \times 10^4$ 0.35 M H_2O	0.48 M H_2O	0.69 M H_2O
0.0		$\leq 10^{-4}$	
4.00	1.34	1.29	
6.00	1.70	1.94	2.01
8.00	1.54	2.64	2.46
10.0	1.47	2.68	3.42
12.5	1.50	2.55	3.99
15.0	1.50	2.32	4.17
17.5	1.44	2.10	3.39
20.0	1.35	1.80	2.40

[a] Solubility of vitamin B_{12a} in water $\approx 8.0 \times 10^{-2}$ M.

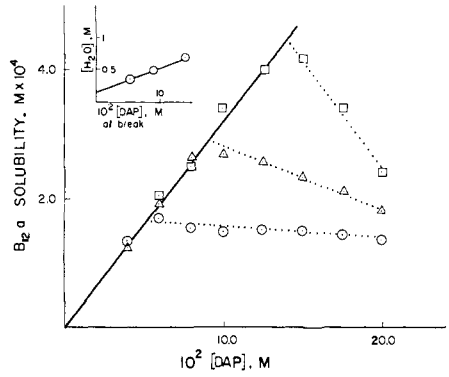

Figure 2. Plot of solubility of vitamin B_{12a} in benzene in the presence of DAP *vs.* stoichiometric DAP concentration: (○) 0.35 M, (△) 0.48 M, and (□) 0.69 M water.

B_{12a}, and decreasing the amount of water per surfactant will result in decrease of solubilization power. A plot of the water concentration against the concentration of DAP at which the vitamin B_{12a} solubility begins to decrease gives a linear relationship (see insert in Figure 2). The slope of this plot, 4, may be considered as the minimum number of water molecules per surfactant required to keep the solubility dependence of vitamin B_{12a} linear. The solubility data can be also utilized to obtain information on the number of surfactant molecules associated with a cobalamin molecule. The concentration of micelles, [M], is given by[10]

$$[M] = (C_D - CMC)/N \qquad (2)$$

where C_D is the stoichiometric surfactant concentration, CMC is the critical micelle concentration, and N is the number of surfactant molecules forming the aggregate. Since vitamin B_{12a} is completely insoluble in benzene, assuming a 1:1 interaction between the micelle and the cobalamin, its solubility in given surfactant solutions represents the concentration of micelles. Put it in other words, the vitamin B_{12a} is being used to titrate the micelles. From the obtained straight line plot of cobalamin solubility, that is, micelle concentration *vs.* (C_D − CMC), a value of 300 ± 30 is calculated for N. Although the assumptions involved in this calculation may not be entirely valid, the number of surfactant molecules required to solubilize vitamin B_{12a} is, not unexpectedly, considerably greater than the range of aggregation numbers quoted for reversed micelles in nonpolar solvents.[13]

(13) F. M. Fowkes in "Solvent Properties of Surfactant Solutions," K. Shinoda, Ed., Marcel Dekker, New York, N. Y., 1967, p 65.

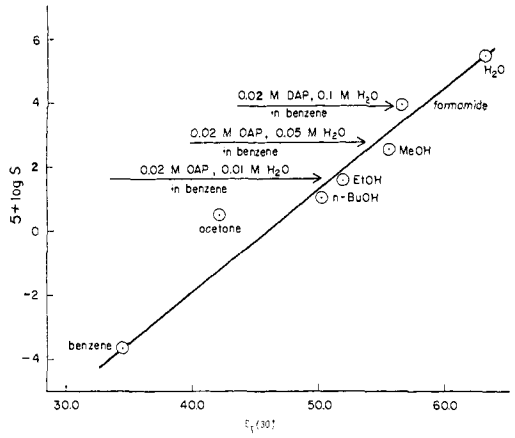

Figure 3. Solubility of glycine in different solvents *vs.* solvent polarity parameter $E_T(30)$.

The solubility of glycine in benzene, 2×10^{-9} *M*, is also increased considerably in the presence of DAP (Table III). Assuming, once again, a 1:1 interaction

Table III. Solubility of Glycine in Benzene in the Presence of DAP at 25.0°[a]

10^2[DAP], *M*	Solubility, $M \times 10^3$
1.00	2.00
2.00	3.74
2.00	0.40[b]
2.00	1.75[c]
2.00	5.70[d]
3.00	4.80
5.00	10.4
7.00	13.3
9.00	17.7
10.0	24.9

[a] Containing 0.10 *M* H_2O, unless stated otherwise. [b] Containing 0.010 *M* H_2O. [c] Containing 0.050 *M* H_2O. [d] Containing 0.20 *M* H_2O.

between DAP and glycine, the latter can be used to titrate the micelle concentration. Plotting the data in Table III according to eq 2 gives a good straight line from which a value of 5 ± 1 is obtained for the aggregation number of DAP. This value agrees well with that obtained previously for neat DAP in benzene by ^1H nmr spectroscopy.[8,14] The validity of using benzene-insoluble substrates to titrate micelle concentrations is therefore substantiated. The implication of these results is that while relatively small molecules do not affect the size of aggregates, larger ones, if solubilized, will associate with increased number of surfactant molecules. Since there is no information, at present, on the shapes of these aggregates, terms "inverted" or "reversed micelles" should be used with caution and only in keeping with the accepted terminology.[13]

The available information on glycine solubilities in water, formamide, methanol, ethanol, 1-butanol, and acetone[15] allows the construction of a linear correlation between the logarithm of glycine solubilities in these solvents and in benzene *vs.* the solvent polarity parameter $E_T(30)$ (Figure 3). Such correlation provides information on the effective microenvironment of DAP-solubilized glycine in benzene. Although other factors, such as hydrogen bonding, need to be considered, once again it is seen (Table III and Figure 3) that the environment of glycine is determined by the concentrations of DAP and water.

Table IV summarizes the dissociation constants of

Table IV. pK_a Values in Different Media

	pK_a			
	In H_2O	In 5.0×10^{-2} *M* CTAB in H_2O	In 5.0×10^{-1} *M* NaLS in H_2O	In 0.20 *M* DAP, 0.10 *M* H_2O in benzene
Vitamin B_{12a}	7.60 (7.64)[a]	7.67	7.65	7.6 ± 0.4
Glycine	9.60	9.60	9.80	
Imidazole	7.06[b]	7.13	7.94	
NaN_3	4.72[c]	4.65	4.82	

[a] Reference 2. [b] Taken from A. E. Martell, "Stability Constants," Part II. The Chemical Society, London, 1964, p 387. [c] Taken from L. G. Sillen, "Stability Constants," Part I, The Chemical Society, London, 1964, pp 116, 160.

vitamin B_{12a} (eq A), imidazole (eq B), glycine (eq C),

$$\text{Bzm-Co-OH}_2 \overset{K_0}{\rightleftharpoons} \text{Bzm-Co-OH} + \text{H}^+ \quad \text{(A)}$$

$$\text{Im}^+ \overset{K_1}{\rightleftharpoons} \text{Im} + \text{H}^+ \quad \text{(B)}$$

$$\text{RNH}_3^+ \overset{K_1}{\rightleftharpoons} \text{RNH}_2 \quad \text{(C)}$$

and sodium azide (eq D) in different environments.

$$\text{HN}_3 \overset{K_1}{\rightleftharpoons} \text{N}_3^- + \text{H}^+ \quad \text{(D)}$$

Rate constants for the equilibrium attainment of cobalamin formation, k_ψ, were determined spectrophotometrically at 350, 357, and 348 nm for Y = glycine, N_3^-, and imidazole, respectively, under pseudo-first-order conditions in the thermostated cell compartments of a Beckman Kintrack VII or a Cary 118C spectrophotometer. Individual k_ψ values were obtained from good linear plots of log $(A_\infty - A_t)$ *vs.* time. Rate constants for the anation, k_1^{app}, and aquation, k_{-1}^{app}, were calculated, with the exception noted below, from the slopes and intercepts of straight line plots of the pseudo-first-order rate constant for equilibrium attainment, k_ψ *vs.* stoichiometric [Y], containing at least four points. Values for k_1^{app} and k_{-1}^{app} are considered to deviate from the mean by 6 and 12%, respectively. In addition to calculating the equilibrium or stability constants from $K_{kin}^{app} = k_1^{app}/k_{-1}^{app}$ they were independently determined from linear plots of log [Y] *vs.* log [Bzm-Co-Y]/[Bzm-Co-OH_2] yielding K_{therm}^{app}. The observed slopes of these plots (0.90–1.05) substantiate the participation of only 1 mol of Y in the equilibria.[16]

Rate constants for the equilibrium attainment of cobalamin formation in aqueous solutions are in Table V and values of k_1^{app} and k_{-1}^{app}, K_{kin}^{app} and K_{therm}^{app} for the interaction of sodium azide, glycine, and imidazole in water and in aqueous micellar CTAB and NaLS are given in Table VI. Rate constants for the

(14) O. A. El Seoud, E. J. Fendler, J. H. Fendler, and R. T. Medary, *J. Phys. Chem.*, 77, 1876 (1973).

(15) A. Seidell, "Solubilities of Organic Compounds," 3rd ed, Vol. II, Van Nostrand, New York, N. Y., 1941, p 122.

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

Table V. Rate Constants for the Equilibrium Attainment of Cobalamin Formation at 25.0° [a]

	$10^4 k_\psi$, sec^{-1}		
	H_2O	5.0×10^{-2} M CTAB in H_2O	5×10^{-1} M NaLS in H_2O
10^3[imidazole], M			
2.00	33.8	48.0	6.50
4.00	82.5	74.4	10.8
6.00	130	117	
8.00	240	248	34.2
10.0	354	372	53.3
12.0	666	477	
16.0	1375	1,484	396
20.0			1108
10^3[NaN_3], M			
1.50		11,600	2960
2.00		17,200	3950
2.50		21,500	5250
3.00		26,600	6170
3.50		34,600	7520
4.00		36,900	9030
10^3[glycine], M			
4.00			0.0055
8.00			0.0085
12.0			0.010
16.0	0.96	0.415	0.0140
24.0	1.18	0.428	0.0185
32.0	1.25	0.480	0.0240
40.0	1.25	0.458	
48.0	1.45	0.545	
56.0		0.571	

[a] Stoichiometric [B_{12a}] = 5.0×10^{-5}; 0.10 M CH_3COONa buffer, pH 6.00.

aquation and anation reactions of vitamin B_{12a} by glycine agree well with those given in the literature.[5,6] Rate constants for the equilibrium attainment of the imidazole adduct of vitamin B_{12a}, k_ψ values, depend linearly on imidazole concentration only in the 2–6 $\times$ 10^{-3} M imidazole range. At higher imidazole concentrations k_ψ increases curvilinearly with increasing imidazole concentrations (Table V). In the linear range, the only range apparently investigated by Randall and Alberty,[1,2] our value of k_1^{app} = 2.1 M^{-1} sec^{-1} (Table VI) agrees well with that determined by them (k_1^{app} = 2.0 M^{-1} sec^{-1}).[1,2] The reported kinetic and thermodynamic parameters for the interaction of imidazole with vitamin B_{12a} in water and in aqueous micellar CTAB and NaLS were obtained, therefore, at imidazole concentration ranges not exceeding 6 $\times$ 10^{-3} M. Tables VII–IX summarize the data for the interaction of glycine, imidazole, and sodium azide with vitamin B_{12a} in benzene in the presence of surfactants. It is seen that the apparent rates depend markedly on the concentrations of surfactant, solubilized water, and ligands. Experimental difficulties (solubility and reactivity) precluded the coverage of a wide range of water and surfactant concentrations for each ligand.

Discussion

Origin of micellar effects can be discussed in terms of substrate partitioning between the micellar and bulk phases and differential reaction rates in these two phases.[17,18] Ligand-exchange reactions in vitamin B_{12a} are equilibrium processes (eq 1). Effects of surfactants need to be separately considered, therefore, on k_1 and k_{-1}. Anation, governed by k_1, in addition to the substrate involves the ligand whose effective concentration and ionization may well be influenced by the surfactants. Aquation, governed by k_{-1}, is more straightforward since it is a pseudounimolecular process. Influence of surfactants on the obtained data, expressed as k_1^{app} and k_{-1}^{app} (Tables VI–IX), can be understood better if information is available on the effects of surfactants on the effective concentrations and ionization of reactants. Solubility and dissociation constant determinations of vitamin B_{12a}, glycine, imidazole, and sodium azide in the different surfactant systems have provided the required information in water. We could not, however, determine the ionization constants of the ligands in the nonaqueous systems meaningfully.

Ligand-Exchange Reactions in Aqueous Micelles. The high water solubility of vitamin B_{12a}, compared to that in benzene, precludes the possibility of its appreciable penetration into the micellar interior. Indeed, based upon the obtained correlation between the absorption maxima of the α and β bands of vitamin B_{12} and solvent polarity parameter $E_T(30)$, the environment of vitamin B_{12} in 5.0 $\times$ 10^{-2} M CTAB is aqueous like (Figure 1). Some interactions, likely to be of electrostatic origin, at the highly charged micellar sodium dodecyl sulfate surface are indicated, however. Glycine, sodium azide, and imidazole each partition favorably into the aqueous phase. Their pK_a values are affected by micellar CTAB and NaLS (Table IV). Conversely, the dissociation constant of vitamin B_{12a} (eq A) is unaffected by micellar surfactants (Table IV).

The proposed mechanism for ligand-exchange reactions in vitamin B_{12a} assumes that binding occurs to aquocobalamin by Y but not by YH^+ (eq 3), *i.e.*, $k_2 \ll k_1$.

$$\begin{array}{ccc} \text{Bzm-Co-OH} + \text{H}^+ & & \\ \uparrow\downarrow K_0 & & \\ \text{Y} + \text{Bzm-Co-OH}_2 & \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} & \text{Bzm-Co-Y} + \text{H}_2\text{O} \\ \uparrow\downarrow K_1 & & \uparrow\downarrow K_2 \\ \text{YH} + \text{Bzm-Co-OH}_2 & \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} & \text{Bzm-Co-YH} + \text{H}_2\text{O} \\ \uparrow\downarrow K_0 & & \\ \text{Bzm-Co-OH} + \text{H}^+ & & \end{array} \qquad (3)$$

$$K_{kin} = k_1/k_{-1}$$

The observed rate constant for the anation at a given pH, k_1^{app}, approximates to

$$k_1^{app} = \frac{k_1}{(1 + K_0/[H^+])(1 + [H^+]/K_1)} \qquad (4)$$

Similarly, K_{therm}^{app}, is expressed by

$$K_{therm} = K_{therm}^{app}(1 + K_0/[H^+])(1 + [H^+]/K_1) \qquad (5)$$

where

$$K_{therm}^{app} = [\text{Bzm-Co-Y}]/[\text{Y}][\text{Bzm-Co-OH}_2] \qquad (6)$$

at a given pH value. The apparent rate constant for aquation, k_{-1}^{app}

$$k_{-1}^{app} = k_{-1} \qquad (7)$$

(17) E. J. Fendler, C. L. Day, and J. H. Fendler, *J. Phys. Chem.*, **76**, 1460 (1972).

(18) E. H. Cordes and R. B. Dunlap, *Accounts Chem. Res.*, **2**, 239 (1969); E. H. Cordes and C. Gitler, *Progr. Bioorg. Chem.*, **2**, 1 (1973).

Table VI. Apparent Kinetic and Thermodynamic Parameters for Ligand Exchange Reactions in Vitamin B_{12a} in Aqueous Solutions at 25.0°[a]

Ligand	Solvent system	k_1^{app}, M^{-1} sec^{-1}	k_{-1}^{app}, sec^{-1}	K_{kin}^{app}, M^{-1}	K_{therm}^{app}, M^{-1}
NaN_3	H_2O[b]	1620	4.4×10^{-2} [c]		3.7×10^4
	5.0×10^{-2} *M* CTAB	889	8.1×10^{-2} [c]		1.1×10^4
	5.0×10^{-1} *M* NaLS	125	2.3×10^{-2} [c]		9.4×10^3
Imidazole	H_2O	2.1	14.2×10^{-4} [c]		1.48×10^3
	5.0×10^{-2} *M* CTAB	1.98	15.1×10^{-4} [c]		1.31×10^3
	5.0×10^{-1} *M* NaLS	0.25	4.47×10^{-4} [c]		5.6×10^2
Glycine	H_2O	1.5×10^{-3}	7.4×10^{-5}	20.3	23.0
	5.0×10^{-2} *M* CTAB	4.17×10^{-4}	3.4×10^{-5}	12.3	30.0
	5.0×10^{-1} *M* NaLS	6.5×10^{-5}	3.6×10^{-6}	21.6	19.0

[a] Stoichiometric [vitamin B_{12a}] = 5.0×10^{-5} *M*; 0.10 *M* CH_3COONa at pH 6.00. [b] Taken from ref 2. [c] Calculated from K_{therm}^{app} and k_1^{app}.

Table VII. Interaction of Glycine with Vitamin B_{12a} in Benzene in the Presence of DAP at 24.8°[a]

		10^2k_ψ, sec^{-1}							
	10^3[DAP], *M*	0.5×10^{-4} *M* glycine	1.0×10^{-4} *M* glycine	1.5×10^{-4} *M* glycine	2.0×10^{-4} *M* glycine	k_1^{app}, M^{-1} sec^{-1}	$10^2k_{-1}^{app}$, sec^{-1}	$10^2k_{-1}^{app}$, sec^{-1} [b]	K_{kin}^{app}, M^{-1}
0.01 *M* H_2O	1.00	1.83	1.92	2.04	2.23	24.0	1.70	1.21	1410
	2.00	2.04	2.20	2.44	2.69	41.0	1.82	1.65	2250
	4.00	2.23	2.51	2.63	2.73	40.0	2.06	1.65	1940
	8.00	2.76	3.20	3.35	3.70	60.0	2.50	2.03	2400
	10.0	3.65	3.93	4.20	4.33	46.0	3.45	2.57	1320
	20.0	3.90	4.52	5.07	5.62	100	3.33	3.37	3000
	25.0	3.30	3.85	4.32	4.95	98.0	2.87	2.45	3400
	50.0	2.57	2.90	3.23	3.52	62.0	2.28	1.24	2700
	75.0	1.70	1.93	2.25	2.60	60.0	1.35	1.08	4430
	100.0	1.43	1.73	1.87	2.38	50.0	1.16		4350
	200.0	0.95	1.12	1.40	1.55	40.0	0.75		5350
0.031 *M* H_2O	5.00	0.79	0.91	0.98	1.11	20.0	0.70		2857
	10.0	1.11	1.28	1.48	1.60	35.0	0.94		3723
	20.0	1.22	1.45	1.62	1.87	40.0	1.04		3846
	25.0	1.03	1.18	1.33	1.46	28.0	0.90		3111
	50.0	0.70	0.77	0.88	0.96	18.0	0.60		3000
	100.0	0.50	0.68	0.73	0.82	15.0	0.52		2885
0.050 *M* H_2O	5.0	0.31	0.33	0.40	0.44	10.0	0.25		4000
	10.0	0.45	0.56	0.61	0.73	17.0	0.37		4595
	20.0	0.52	0.59	0.70	0.80	20.0	0.40		5000
	25.0	0.39	0.50	0.58	0.62	14.5	0.35		4143
	50.0	0.25	0.26	0.31	0.38	8.00	0.20		4000
	100.0	0.16	0.16	0.20	0.22	5.00	0.13		3846
0.10 *M* H_2O	10.0	0.17	0.21	0.23	0.25	5.25	0.15		3500
	20.0	0.23	0.26	0.28	0.32	6.00	0.20		3000
	25.0	0.26	0.27	0.31	0.35	6.75	0.21		3214
	50.0	0.22	0.23	0.27	0.31	6.50	0.18		3611
	75.0	0.18	0.20	0.25	0.27	6.00	0.15		4000
	100.0	0.16	0.18	0.21	0.24	5.75	0.13		4423
1.32 *M* H_2O, 0.20 *M* DAP[c]									
10^2[glycine]		0.92	1.17	1.56	1.95	2.34	3.13	3.91	
10^4k_ψ, sec^{-1}		7.00	10.5	13.8	15.3	17.9	23.8	32.5	
$k_1^{app} = 7.4 \times 10^{-2}$ M^{-1} sec^{-1}; $k_{-1}^{app} = 2 \times 10^{-4}$ sec^{-1}; $K_{kin}^{app} = 367$ M^{-1}									

[a] Stoichiometric vitamin B_{12a} concentration = 5.0×10^{-7} *M*, unless stated otherwise. [b] Obtained by following the decay of the isolated complex of vitamin B_{12a}. [c] [Vitamin B_{12a}] = 5.0×10^{-5} *M*.

Substituting the obtained dissociation constants of vitamin B_{12a}, K_0, and those for glycine, imidazole, and sodium azide, K_1, in the different media into eq 4 and 5, rate and equilibrium constants for ligand exchange reactions in vitamin B_{12a} are calculated (Table X). Data for the interaction of imidazole with vitamin B_{12a} in water agree well with that available in the literature (Table X). Effects of micelles on these processes are relatively meager and can largely be rationalized by electrostatic considerations. In the case of imidazole, micellar effects on anation can be rationalized on the influence of surfactants on the dissociation constant of imidazole (Tables IV, VI, and X). Rate constants for the anation, k_1^{app}, are decreased by anionic micellar NaLS, and with the exception of that on the interaction of glycine with vitamin B_{12a}, they are unaffected by CTAB. Zwitterionic glycine, present at the pH of the experiment, is likely to facilitate electrostatic interactions both with cationic and anionic surfactants, reducing thereby the effective glycine concentration. Rate constants for the aquation, k_{-1}^{app}, may well involve the equilibria between Bzm–Co–Y and Bzm–Co–YH, governed by K_2 (eq 3). Equation 7 is inadequate in this case and should be substituted by

$$k_{-1}^{app} = \frac{k_{-1} + k_{-2}[H^+]/K_2}{1 + [H^+]/K_2} \quad (8)$$

Table VIII. Interaction of Imidazole with Vitamin B_{12a} in Benzene in the Presence of Surfactants at 25.0°[a]

Surfactant	H_2O, M	10^5[imidazole], M	10^4k_ψ, sec^{-1}	k_1^{app}, M^{-1} sec^{-1}	k_{-1}^{app}, sec^{-1}	K_{kin}^{app}, M^{-1}	k_{therm}^{app}, M^{-1}
0.20 M DAP	8.9×10^{-2}	40.0	10.7				
		50.0	12.2				
		60.0	13.8				
		70.0	15.6				
		80.0	17.2	1.6	4.0×10^{-4}	4.1×10^3	3.6×10^3
0.20 M DAP	1.10	2.00	5.44				
		5.00	7.75				
		7.50	9.20				
		10.0	10.7	6.7	4.3×10^{-4}	1.5×10^4	1.1×10^4
0.02 M DAP[b]	8.9×10^{-2}	1.00	77.4				
		2.00	46.6				
		3.00	31.4				
		4.00	18.8				
		5.00	15.7				
		5.5	28.4				
		6.0	12.3				
		10.0	11.5				
		20.0	9.29	1200[c]	10.8[d]		1.3×10^4
0.20 M Aerosol-OT	1.10	5.0	1.70				
		10.0	2.60				
		15.0	3.10				
		30.0	5.60				
		50.0	8.50	1.7	1.0×10^{-4}	1.7×10^4	2.2×10^4

[a] Stoichiometric [vitamin B_{12a}] = 5.0×10^{-6} M, unless stated otherwise. [b] Stoichiometric [vitamin B_{12a}] = 1.0×10^{-6} M. [c] See Results and Discussion. [d] Calculated from K_{therm}^{app} and k_{-1}^{app}.

Table IX. Interaction of Sodium Azide with Vitamin B_{12a} in Benzene in the Presence of 0.20 M DAP at 25.0°[a]

10^5[NaN_3], M	10^4k_ψ, sec^{-1}
3.00	5.20
5.00	8.70
5.70	9.80
6.00	11.2
7.00	12.6
8.50	15.5
10.0	17.5

$k_1^{app} = 16.3\ M^{-1}\ sec^{-1}$
$k_{-1}^{app} = 2.0 \times 10^{-5}\ sec^{-1}$
$K_{kin}^{app} = 8.0 \times 10^5\ M^{-1}$
$K_{therm}^{app} = 6.2 \times 10^5\ M^{-1}$

[a] Combining 2.2×10^{-2} M H_2O. Stoichiometric [vitamin B_{12a}] = 5.0×10^{-6} M.

The obtained data, however, are not sufficiently detailed or accurate to calculate micellar effects on K_2. The general trend is analogous to that observed on k_1^{app}. The most pronounced effect is the 19-fold decrease of the aquation rate of the glycine complex of vitamin B_{12a} by micellar NaLS. Cationic micellar CTAB enhances modestly the aquation of the azide complex, has no effect on that of the imidazole complex, and decreases that of the glycine complex (Table X). The overall effects of aqueous micelles on the binding of ligands to vitamin B_{12a}, K_{therm}^{app}, are even less significant (Table X).

Ligand-Exchange Reactions in Restricted Polar Media in Benzene. The most remarkable observation of this study is that such a large molecule as vitamin B_{12a} can be solubilized, together with some water molecules, in benzene by dodecylammonium propionate and Aerosol-OT. In this environment cobalamin, contained in restricted water pools, is surrounded by some 300 surfactant molecules and is, therefore, effectively shielded from the bulk apolar solvent. A similar situation has been observed in the solubilization of hemin in benzene.[19] Equally significant is that by varying the concentrations of substrate and water as solubilizates, the apparent polarity of vitamin B_{12a} can be affected. Thus, using the absorption maxima *vs.* $E_T(30)$ correlation the effective environment of aquocobalamin can be seen to resemble pyridine or highly structured water (Figure 1 and Table I). Effects of polarity restrictions on rates and equilibria of reaction 1 can therefore be investigated.

The main body of information has been obtained using glycine as ligand (Table VII). It is seen (Table III and Figure 3) that glycine is completely localized in the polar interior of the surfactant aggregates. Its effective concentration may, therefore, be equated with that in the micellar phase. Although such an approach may lead to successful resolution of effects on bimolecular processes in aqueous micellar solutions, in nonpolar systems concentrations of solubilized water, and hence the size and apparent polarity of pools, need also to be considered. At present we do not have means for the treatment of this complicated partitioning problem. Although not ideal, glycine (as well as sodium azide and imidazole) concentrations have been expressed stoichiometrically, *i.e.*, that dissolved in the surfactant–benzene–water solutions and rates of anation are only given therefore as k_1^{app}. This allows intercomparison among the different systems quantitatively. The biggest effect on rates and equilibrium constants in the anation of vitamin B_{12a} by glycine occurs, as expected, at the surfactant–benzene system which contains the smallest possible water concentration, 0.01 M H_2O, in 0.02 M DAP (Table VII). Here the apparent environment of vitamin B_{12} resembles that of highly structured water (Table I, Figure 1) while that for glycine approximates the polarity of alcohols (Figure 3). Differences in the apparent environments of the reactants in surfactant aggregates are not unexpected. Indeed, it has been demonstrated that dynamic solubilization sites of

(19) W. Hinze and J. H. Fendler, unpublished results.

Table X. Kinetic and Thermodynamic Parameters for Ligand-Exchange Reactions in Vitamin B_{12a} in Aqueous Solutions at 25.0°

Ligand	Solvent system	k_1, M^{-1} sec^{-1} [a]	k_{-1}, sec^{-1} [b]	K_{therm}, M^{-1} [c]
NaN_3	H_2O	1730[d]	4.3×10^{-2} [d]	4.0×10^4 [d]
		1200[e]	2.9×10^{-2} [e]	
	5.0×10^{-2} M CTAB	946	8.0×10^{-2}	1.2×10^4
	5.0×10^{-1} M NaLS	235	2.3×10^{-2}	1.0×10^4
Imidazole	H_2O	26.9 (27 ± 6)[d]	1.4×10^{-3}	1.9×10^4
	5.0×10^{-2} M CTAB	29.4	1.5×10^{-3}	1.9×10^4
	5.0×10^{-1} M NaLS	22.5	4.5×10^{-4}	5.0×10^4
Glycine	H_2O	6.08	6.45×10^{-5}	9.4×10^4
	5.0×10^{-2} M CTAB	1.68	1.39×10^{-5}	1.2×10^5
	5.0×10^{-1} M NaLS	0.41	3.40×10^{-6}	1.2×10^5

[a] Calculated using eq 4 and the dissociation constants in Table IV. [b] Calculated from K_{therm} and k_1 values. [c] Calculated using eq 5. [d] Taken from ref 2. [e] Taken from ref 3.

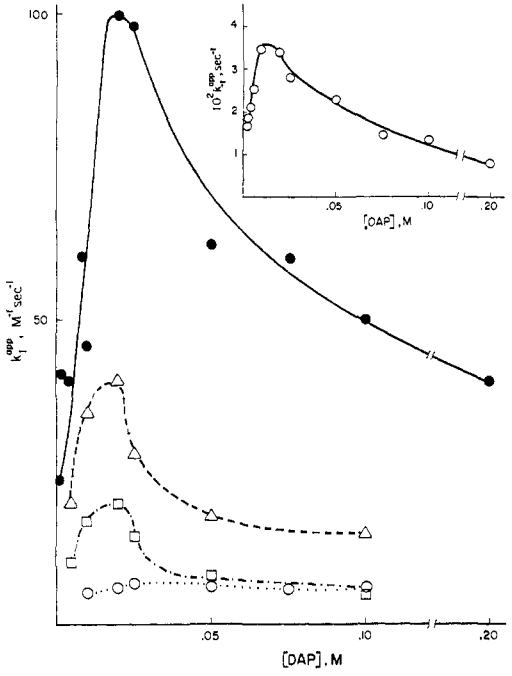

Figure 4. Plot of k_1^{app} *vs.* DAP concentration for the interaction of vitamin B_{12a} with glycine in benzene in the presence of (●) 0.010 M, (△) 0.031 M, (□) 0.50 M, and (○) 0.10 M solubilized water at 25.0°. Insert shows a plot of k_{-1}^{app} *vs.* stoichiometric DAP concentration for the same reaction in benzene in the presence of 0.010 M solubilized water.

reactant, transition states, and products in micellar systems are different.[20]

Although the meaning of such comparison may be questionable (*vide supra*), the apparent rate constant for the anation of vitamin B_{12a} by glycine in benzene in the presence of 0.01 M water and 0.02 M DAP, 100 M^{-1} sec^{-1}, is some 66,000-fold faster than that in pure water ($k_1^{app} = 1.5 \times 10^{-3}$ M^{-1} sec^{-1}, Table VI). Similarly, k_{-1}^{app} in the restricted environment of the water pool, 3.33×10^{-2} sec^{-1}, is some 445-fold faster than in water, 7.4×10^{-5} sec^{-1}. Rate profiles for the aquation and anation are similar. At constant water concentration, increasing the surfactant concentration in benzene results in increased values of k_1^{app} and k_{-1}^{app} up to a maximum, after which the rates decrease logarithmically (Figure 4). These results parallel those obtained from the solubility measurements of vitamin B_{12} (Figure 2). Increasing surfactant concentration causes increasing solubilization and rate enhancement up to a maximum, after which the number of water molecules per surfactant decreases with resultant decrease in solubilization and rate enhancement. At the highest surfactant and water concentrations in benzene the observed rate constants ($k_1^{app} = 7.4 \times 10^{-2}$ M^{-1} sec^{-1}; $k_{-1}^{app} = 2.0 \times 10^{-4}$ sec^{-1}, Table VII) almost approach those in pure water. The significant increase in rate constants for anation and aquation of vitamin B_{12a} at 0.02 M DAP concentration in benzene with decreasing amounts of solubilized water (Figure 4) parallels the increase in the apparent microscopic polarity of vitamin B_{12} (Table I). One may speculate, therefore, that the observed rate enhancements are largely due to tightening of the solvation shell around the reagents.

Values for k_{-1}^{app}, obtained by following the decay of the glycine complex of vitamin B_{12a} (Table VII) in benzene in the presence of 0.01 M H_2O, have been utilized to estimate the binding constant, K, between DAP and vitamin B_{12a} using eq 9 where $(k_{-1}^{app})_\psi$ and

$$\frac{1}{(k_{-1}^{app})_\psi} = \frac{1}{(k_{-1}^{app})_m} + \left(\frac{1}{(k_{-1}^{app})_m}\right)\left(\frac{1}{C_D - CMC}\right)\frac{N}{K} \quad (9)$$

$(k_{-1}^{app})_m$ are the apparent first-order rate constants for the decomposition of the glycine adduct at a given surfactant concentration and that in the micellar phase, respectively. From the obtained straight line, on plotting the left-hand side of eq 9 *vs.* $1/(C_D - CMC)$, a value of 570 is calculated for K/N. Using 300 as the aggregation number of dodecylammonium propionate, the value for K, 1.7×10^5 M^{-1}, indicates significant binding. The intercept of the plot, according to eq 9, gives $(k_{-1}^{app})_m = 3.0 \times 10^{-2}$. This value represents the rate in the micellar environment and is in good agreement with that obtained as the maximum in the kinetic rate profile (Table VII). Binding constants of similar magnitude have been obtained from the data on the anation (k_1^{app}).

Although imidazole does not dimerize in benzene under our concentration range,[21] it is soluble in benzene and consequently does not bind strongly to DAP.[22] Not unexpectedly, therefore, its interaction with vitamin B_{12a} in water solubilized by 0.20 M DAP or Aerosol-OT

(20) E. H. Fendler, C. L. Day, and J. H. Fendler, *J. Phys. Chem.*, **76**, 1960 (1972).

(21) W. Huckel, J. Datow, and E. Simmersbach, *Z. Phys. Chem., Abt. A*, **186**, 129 (1940).

(22) O. A. El Seoud, E. J. Fendler, and J. H. Fendler, *J. Chem. Soc., Faraday Trans. 1*, **70**, 459 (1974).

is not significantly different from that in pure water (Table VIII). More dramatic effects are observed, however, when 8.9×10^{-2} M water is solubilized by 2.0×10^{-2} M DAP in benzene. In this system rate constants for the equilibrium attainment of reaction 1, k_ψ, do not increase linearly with increasing imidazole concentration but rather decrease logarithmically (Figure 5). At low imidazole concentration the advantageous proximity to vitamin B_{12a} is maximized. At the lowest stoichiometric imidazole concentration (1.0×10^{-5} M) the rate constant for the anation of vitamin B_{12a} in the presence of 2.0×10^{-2} M DAP in benzene is 353-fold faster than that in water. The 25,200-fold increase in the aquation rate is even more impressive. The more favorable destabilization of imidazole cobalamin as compared to vitamin B_{12a}, coupled with the considerably increased effective water concentration in the tight micellar cavity, is responsible for the more pronounced enhancement of k_{-1}^{app} as compared to k_1^{app}. Increasing concentrations of imidazole decrease the amounts of imidazole per micelle with resultant saturation kinetics with respect to imidazole.

Values of k_1^{app} and k_{-1}^{app} for the equilibrium formation of Bzm-Co-N_3 in the reversed micellar system are factors of 99 and 2100 times slower than those in pure water (Table IX). Alteration of the ionization equilibria, hydrogen bonding, and changes in the effective water and reagent activities is likely to be responsible for these effects.

The nature of the ligand and the size of the surfactant mantle and of solubilized water pool clearly influence anation and aquation rates of surfactant solubilized cobalamins in benzene. This type of restricted polar environment for bimolecular reactions involving relatively large substrates has pronounced effects on the reaction rates and, considering both the anation and aquation reactions, exhibits considerable specificity. Encouraged by the versatility of our model system we are currently investigating rates and equilibrium constants for a variety of cobalamin reactions. Particularly, we hope to unravel the binding of more complex amino acids and peptides in restricted polar environments and relate the obtained information to vitamin B_{12} dependent reactions and to membrane transport processes *in vivo*.

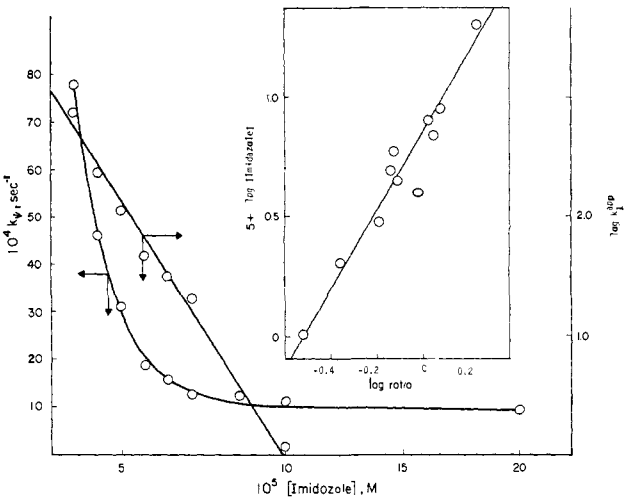

Figure 5. Plots of k_ψ or log k_1^{app} *vs.* imidazole concentration for the reaction of imidazole with vitamin B_{12a} in benzene in the presence of 0.02 M DAP, containing 8.9×10^{-2} M water. Insert shows a plot of log [imidazole] *vs.* log [Bz-Co-imidazole]/[Bzm-Co-OH_2] for the same system.

Acknowledgments. This work was supported by the National Science Foundation and by the Robert A. Welch Foundation.

Communications to the Editor

Kinetic Energy Release in Relation to Symmetry-Forbidden Reactions

Sir:

The concept of orbital symmetry conservation[1] has greatly advanced our understanding of numerous concerted reactions. It has been our aim to use this concept to develop our knowledge of the mechanisms *via* which positive ions undergo unimolecular reactions. Such unimolecular reactions are of course most conveniently examined in the mass spectrometer and, in particular, when these reactions are investigated through the observation of metastable peaks, the range of unimolecular rate constants is reasonably defined (k = 10^4 to 10^6 sec^{-1}) and the nonfixed energy in the transition state is relatively small.[2,3] In the most favorable cases, the nonfixed energy in the transition state is so small that extremely large isotope effects are observed when there is competition between C–H and C–D cleavage. For example, in metastable transitions, the molecular ion of CH_3CD_3 eliminates H in preference to D in the ratio 600:1.[4]

The reactions we have considered all correspond to loss of molecular hydrogen and are summarized in eq 1–5.

$$CH_3CH_3^{\cdot+} \longrightarrow CH_2CH_2^{\cdot+} + H_2 \quad (1)$$

$$CH_2{=}\overset{+}{O}H \longrightarrow HC{\equiv}O^+ + H_2 \quad (2)$$

$$CH_2{=}\overset{+}{N}H_2 \longrightarrow HC{\equiv}\overset{+}{N}H + H_2 \quad (3)$$

$$CH_2{=}\overset{+}{S}H \longrightarrow HC{\equiv}\overset{+}{S} + H_2 \quad (4)$$

$$CH_3{-}\overset{+}{N}H_2 \longrightarrow CH_2{=}\overset{+}{N}H + H_2 \quad (5)$$

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