identified. The presence of a free carboxyl group is a further indication that there should be five free basic groups. Further experiments on the structures of edeines A and B are in progress.

Acknowledgment

We are grateful to Dr. Tessa Abramsky and Dr. Edward L. Tatum for helpful discussions and to Mr. D. Rigakos and Mr. T. Bella for the microanalyses.

References

Battersby, A. R., and Craig, L. C. (1951), *J. Am. Chem. Soc. 73*, 1887.
Borowski, E., Chmara, H., and Jareczek-Morawska, E. (1966), *Biochim. Biophys. Acta 130*, 560.
Dubin, D. T. (1960), *J. Biol. Chem. 235*, 783.
Fraenkel-Conrat, H., Harris, J. I., and Levy, A. L. (1955), *Methods Biochem. Anal. 2*, 359.
Hettinger, T. P., and Craig, L. C. (1968), *Biochemistry 7*, 4147 (this issue; preceding paper).
Hunter, M. J., and Ludwig, M. L. (1962), *J. Am. Chem. Soc. 84*, 3491.
Klee, W. A., and Richards, F. M. (1957), *J. Biol. Chem. 229*, 489.
Kurylo-Borowska, Z. (1967), *Antibiotics 2*, 446.
Kurzer, F., and Lawson, A. (1954), *Org. Syn. 34*, 67.
Poduška, K., Katrukha, G. S., Silaev, A. B., and Rudinger, J. (1965), *Coll. Czech. Chem. Commun. 30*, 2410.
Robin, Y., Audit, C., and Landon, M. (1967), *Comp. Biochem. Physiol. 22*, 787.
Robin, Y., and van Thoai, N. (1961), *Compt. Rend. 252*, 1224.
Roncari, G., Kurylo-Borowska, Z., and Craig, L. C. (1966), *Biochemistry 5*, 2153.
Scanes, F. S., and Tozer, B. T. (1956), *Biochem. J. 63*, 282.
Silaev, A. B., Baratova, L. A., and Katrukha, G. S. (1966), *J. Chromatog. 24*, 61.
Tabor, H., Rosenthal, S. M., and Tabor, C. W. (1958), *J. Biol. Chem. 233*, 907.

The Photolability of Co-alkylcobinamides*

W. H. Pailes and H. P. C. Hogenkamp

ABSTRACT: The spectral properties and photodecomposition of N^5-methyltetrahydrofolate–homocysteine transmethylase suggest that in the enzyme-bound cobalamin the coordinate linkage between cobalt and the 5,6-dimethylbenzimidazole moiety is broken. Thus it would be expected that enzyme-bound cobalamin should react like a cobinamide rather than a cobalamin. Methylcobinamide–ligand complexes were used as model systems to investigate the influence of ligands on the spectrum of methylcobinamide and on the photodecomposition of the carbon–cobalt bond. In general the formation constants of the alkylcobinamide–ligand complexes are very small compared with those of the cyanocobinamide–ligand complexes as a result of the strong electron-donating influence of the alkyl ligands. The formation constant of the *n*-propylcobinamide–imidazole complex was found to be two orders of magnitude smaller than the formation constant of the methylcobinamide–imidazole complex, reflecting the positive inductive effect of the extra ethyl group. Whereas these and earlier results suggest that displacement of water coordinated to cobalt by other bases renders the carbon–cobalt bond more susceptible to photolytic cleavage, the displacement of water by imidazole, 1-methylimidazole, and ammonia were found to greatly decrease the rate of photolysis.

These results have been used to explain the spectral properties and the unexpected light stability of the carbon–cobalt bond of methylated N^5-methyltetrahydrofolate–homocysteine transmethylase, as well as the spectral properties and photolability of propylated transmethylase.

N^5-Methyltetrahydrofolate–homocysteine transmethylase has been highly purified from *Escherichia coli* B and has been shown to contain aquocobalamin as a prosthetic group (Taylor and Weissbach, 1967b). When this purified transmethylase is incubated with [^{14}C]methyltetrahydrofolate in the presence of $FMNH_2$, dithiothreitol, and a catalytic amount of *S*-adenosyl-L-methionine, ^{14}C-labeled holoenzyme is formed (Taylor and Weissbach, 1967a). Subsequent incubation of this labeled enzyme with homocysteine yields ^{14}C-labeled methionine. Taylor and Weissbach (1968) showed conclusively that in the methylated holoenzyme the methyl group is linked to the cobalt atom of the cob-

* From the Department of Biochemistry, The University of Iowa, Iowa City, Iowa 52240. *Received July 25, 1968.* Supported by U. S. Public Health Service Research Grant AM-08627 from the National Institutes of Health.

amide. However, the carbon–cobalt bond of the methylated holoenzyme is not cleaved by light, in contrast to that of methylcobalamin (Hogenkamp, 1966). On the other hand, inactive propylated holotransmethylase, formed by treating reduced holoenzyme with propyl iodide, can be activated by exposure to light (Taylor and Weissbach, 1967c).

Although Taylor and Weissbach (1968) isolated methylcobalamin from denaturated methylated transmethylase the absorbance spectrum of the native holoenzyme is quite different from that of methylcobalamin (Taylor and Weissbach, 1966). The spectrum of the native holoenzyme in the visible region is similar to that of cob(II)alamin,[1] but Taylor and Weissbach (1967b) reported in a subsequent paper that the holoenzyme does not have an eight-line electron spin resonance spectrum and is not oxidized by oxygen, indicating the absence of paramagnetic Co^{2+}. The possibility that the cobalt atom is linked to a sulfhydryl group in the protein (Taylor and Weissbach, 1968) seems unlikely since cobalamin–thiol complexes are known to react readily with alkyl halides to form alkylcobalamins, whereas propylation of the holoenzyme with propyl iodide required reduction with both dithiothreitol and $FMNH_2$. More recently Ertel *et al.* (1968) have isolated sulfitocobalamin from holotransmethylase. However, this cobalamin only accounted for 50% of the total cobamide of the enzyme preparation.

The absorbance maxima of the holoenzyme (355, 405, 445, and 470 mμ) as well as the shift of these maxima to lower wavelengths on propylation lead to the suggestion that the holoenzyme and propylated holoenzyme contain a cobalamin in which the 5,6-dimethylbenzimidazole moiety is not cooordinated to the cobalt atom (Firth *et al.*, 1967; Hogenkamp, 1968). In that case the corrinoid prosthetic group of holotransmethylase would be expected to behave like a cobinamide rather than a cobalamin, and its properties should be comparable with those of the cobinamides.

This communication describes the spectral properties of organoaquocobinamides and of organocobinamides in which the water has been displaced by other ligands. The photolability of these organocobinamides and the effect of alteration of the ligands on the rate of photodecomposition have also been studied.

Experimental Procedure

Materials

Cyanocobalamin was purchased from Sigma Chemical Co. Phosphocellulose (0.94 mequiv/g) was obtained from Schleicher & Schuell. Other chemicals were also purchased from commercial sources. Aquocobalamin was prepared from cyanocobalamin according to Hogenkamp and Rush (1967). Diaquocobinamide was prepared from aquocobalamin by the procedure of Friedrich and Bernhauer (1956), modified as follows: a reaction mixture containing 800 mg of aquocobalamin, 60 ml of 0.33 M cerous nitrate, and 50 ml of 1 N NaOH was heated for 2.5 hr in a boiling-water bath. The solution was then cooled and filtered through Celite, and the cobinamide was desalted by phenol extraction (Barker *et al.*, 1963). The concentrated aqueous solution (1–2 ml) was applied to a 128 × 1.2 cm column of Sephadex G-10, which had been equilibrated with distilled water. The sample was eluted with water at a flow rate of approximately 10 ml/hr and the effluent was collected in 2-ml fractions. Diaquocobinamide was eluted in a prominent peak, detected by its absorbance at 350 mμ between fractions 25 and 40; a minor component was eluted between fractions 10 and 24. The fractions containing diaquocobinamide were pooled and evaporated to dryness yielding 382 mg of a red-orange glass.

Methylcobinamide and *n*-propylcobinamide were prepared by treating diaquocobinamide reduced with chromous chloride in 0.1 M sodium EDTA buffer (pH 9.5) with the corresponding alkyl halides. The reaction products were desalted by phenol extraction and adsorbed on separate columns of phosphocellulose (30 × 2 cm). The columns were washed with water and the alkylcobinamides eluted with 0.025 M ammonium acetate buffer (pH 4.3). Both cobinamides were again desalted by phenol extraction and isolated as a glass.

The purity of the cobinamide preparations was determined by paper chromatography in three solvent systems. Chromatography was performed by the descending technique on Whatman No. 40 paper using the following solvent systems: solvent I, *sec*-butyl alcohol–acetic acid–water (100:1:50); solvent II, *n*-butyl alcohol–isopropyl alcohol–acetic acid–water (100:70:1:100); and solvent III, isopropyl alcohol–NH_4OH–water (7:1:2). R_F values of the cobinamides are presented in Table I.

TABLE I: R_F Values of Cobinamides.

Ligand[a]		Solvent		
Y	Z	I	II	III
H_2O	H_2O	0.42	0.57	0.18
H_2O	CH_3^-	0.50	0.57	0.46
H_2O	$CH_3CH_2CH_2^-$	0.59	0.62	0.53

[a] The ligand in the lower axial coordination site (the propionamide side of the ring) is defined as ligand Y, and the ligand in the upper coordination site (the acetamide side of the ring) is defined as ligand Z.

Absorption spectra were recorded with a Cary Model 15 spectrophotometer using matched 1-cm quartz cells. Other visible and ultraviolet spectral measurements were made with a Zeiss PMQII spectrophotometer.

Concentrations of methyl- and propylcobinamide were determined by comparing the absorbance at 460 mμ of an aqueous solution of the cobinamide with that of a solution of methylcobalamin of known

[1] Cob(II)alamin, reduced aquocobalamin containing divalent cobalt.

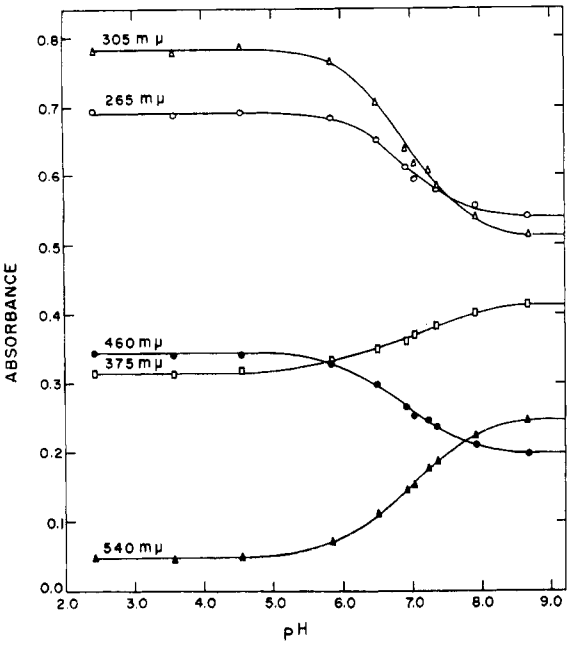

FIGURE 1: Influence of pH on the methylcobinamide–imidazole complex. Each cuvet contained 4.15 × 10^{-5} M methylcobinamide in 0.5 M imidazole adjusted to the desired pH with 1 M phosphate buffer.

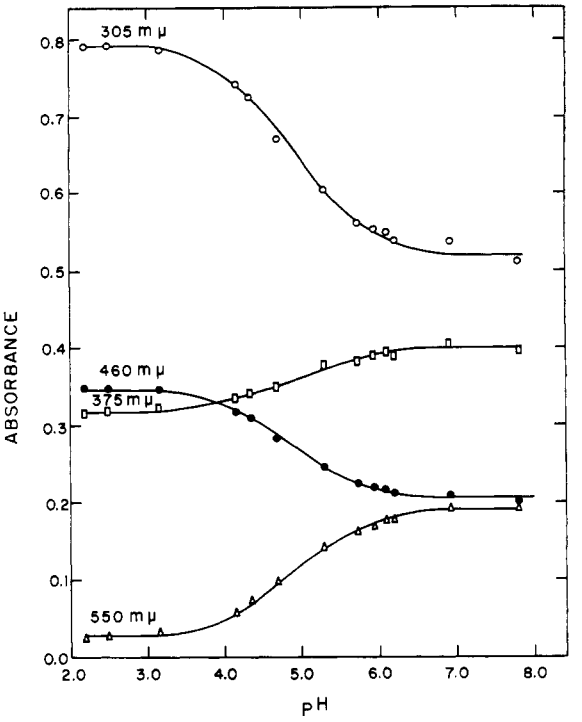

FIGURE 2: Influence of pH on the methylcobinamide–pyridine complex. Each cuvet contained 4.15 × 10^{-5} M methylcobinamide in 0.5 M pyridine adjusted to the desired pH with 1 M phosphate buffer.

concentration in 0.1 N HCl. Methylcobalamin concentration was determined from its absorbance at neutral pH and the molar extinction coefficient of 7.6 × 10^3 M^{-1} cm^{-1} at 525 mμ (Johnson *et al.*, 1963).

The photolysis of aqueous solutions of the cobinamides was performed in 3-ml quartz cuvets exposed to a 200-W tungsten lamp at a distance of 60 cm. First-order rate constants were determined by the method of Guggenheim (1926).

For the substitution of coordinated water or methyl- and propylcobinamide by various ligands, L, the formation constant, K, is defined as K = [L–Co–R]/[L][H_2O–Co–R]. The concentration of water is neglected. Because the cobinamide concentration is extremely small compared with that of the ligand, no correction was made for the amount of bound ligand and [L] corresponds to the total ligand concentration. Since the formation constants have low values, end points, corresponding to 100% formation of the complexes, could not be obtained. The formation constants of the alkylcobinamide–ligand complexes were determined by plotting the reciprocal total ligand concentration against the reciprocal of the optical density change. The intercept on the abscissa corresponds to the negative formation constant.

Results

Formation Constants and Spectra of the Alkylcobinamide–Ligand Complexes. The formation constants and the position of the main absorption bands of the alkylcobinamide–ligand complexes are shown in Table II. Several other possible ligands of biochemical interest such as cysteine, methionine, tyrosine, lysine, and caprolactam were tested for their ability to form complexes with methylcobinamide. However, no changes in the absorption spectrum of methylaquocobinamide in the presence of 1–2 M ligand could be observed. Furthermore no spectral evidence for the formation of a thiocyanate–methylcobinamide complex could be detected. In alkali the spectrum of methylcobinamide moves to shorter wavelengths with new absorption maxima at 453, 362, and 267 mμ, but unfortunately under these conditions (1 N NaOH) methylcobinamide undergoes irreversible changes. For the same reason the affinity of mercaptide ion for methylcobinamide could not be established although in the presence of 2 M mercaptoethanol and 2 M NaOH the spectrum of methylcobinamide showed new maxima at 575, 482, and 365 mμ.

In Figures 1 and 2 is shown the effect of changes in pH on the spectra of the methylcobinamide–imidazole complex and the methylcobinamide–pyridine complex, respectively. At pH values where the ligands are protonated the spectra of these complexes are identical with the spectrum of methylaquocobinamide. The midpoints of these curves are at pH 6.9 and 4.9 for the methylcobinamide–imidazole and the methylcobinamide–pyridine complexes, respectively.

Effect of Ligands on the Photolysis of Methylcobalamin and Methylcobinamide. In acid solution the 5,6-dimethylbenzimidazole moiety of methylcobalamin is protonated and no longer coordinated to cobalt; its position on the cobalt atom is taken up by water. In Figure 3 is shown the effect of pH on the rate of photolysis of the carbon–cobalt bond of methylcobalamin. The apparent pK_a value of methylcobalamin calculated from these data is 2.7. In contrast the rate of photolytic

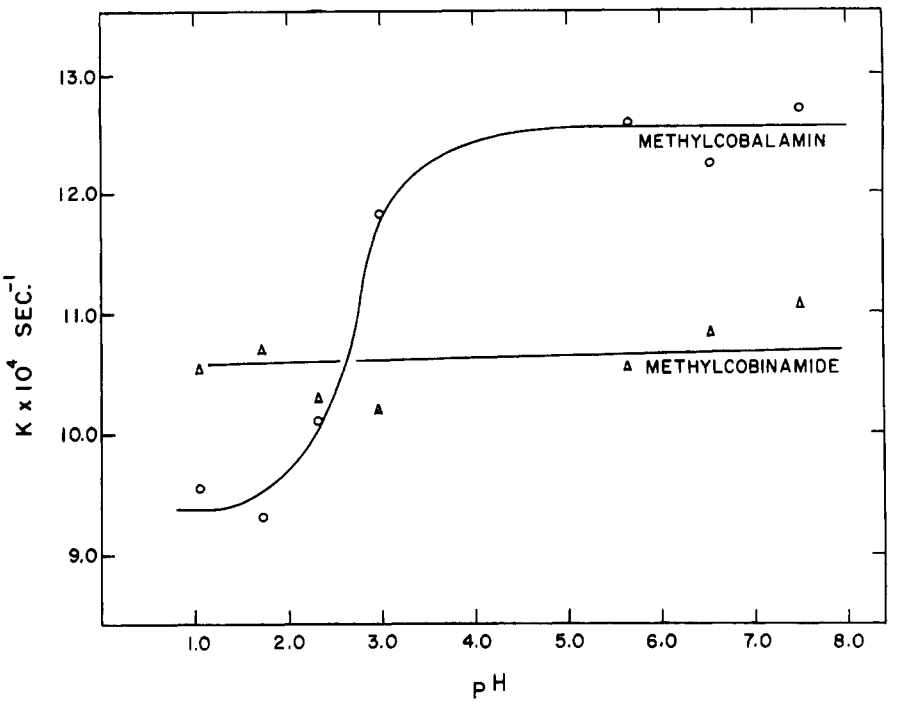

FIGURE 3: Influence of pH on the photolysis of methylcobinamide and of methylcobalamin. Each cuvet, containing 4.32×10^{-5} M methylcobalamin or 4.15×10^{-5} M methylcobinamide in 0.1 M buffer, was exposed to a 200-W tungsten lamp at a distance of 60 cm. The first-order rate constants were determined by the method of Guggenheim (1926).

TABLE II: Formation Constants, K, and Spectral Properties of the Alkylcobinamide–Ligand Complexes.

Ligand[a]		pK_a of						
Y	Z	Ligand Y	K (M^{-1})	λ (mμ)				
H_2O	CH_3^-			461	375		306	264
Cyanide	CH_3^-	9.14	230	572	391		326	271
Imidazole	CH_3^-	6.95	11	524	376	341	307	267
Pyridine	CH_3^-	5.19	6	515	376	340		
1-Methylimidazole	CH_3^-	7.2	5	522	376	341		
Ammonia	CH_3^-	9.25	0.1	515	373	339	306	266
Ethanolamine	CH_3^-	9.44	0.03	510	375	340	306	
Piperidine	CH_3^-	11.22	<0.01	472	372		306	
H_2O	$CH_3CH_2CH_2^-$			445	380		304	263
Imidazole	$CH_3CH_2CH_2^-$	6.95	0.1	495	380	347		

[a] See Table I.

cleavage of the organometallic bond of methylaquocobinamide is not influenced by changes in pH.

The effect of displacing the bound water of methylaquocobinamide by imidazole and ammonia on the rate of photolysis is shown in Figure 4 and 5. The effect of 1-methylimidazole is identical with that of imidazole. To eliminate the possibility that this decrease in the rate of photolysis was merely due to a shielding effect so that the effective light intensity would be decreased, both experiments were repeated with methylcobalamin. These results, also shown in Figures 4 and 5, clearly indicate that imidazole increases, rather than decreases, the rate of photolysis of methylcobalamin, while ammonia has no appreciable effect on the rate of photolysis. The rate of photodecomposition was also determined in 0.5 M imidazole at different pH values in order to obtain evidence that the rate of photolysis is affected only by cobalt-bound ligand. The results (Figure 6) indicate that protonated imidazole has no effect and that only bound imidazole decreases the rate of photodecomposition.

Cyanide ion and pyridine do not stabilize the carbon-

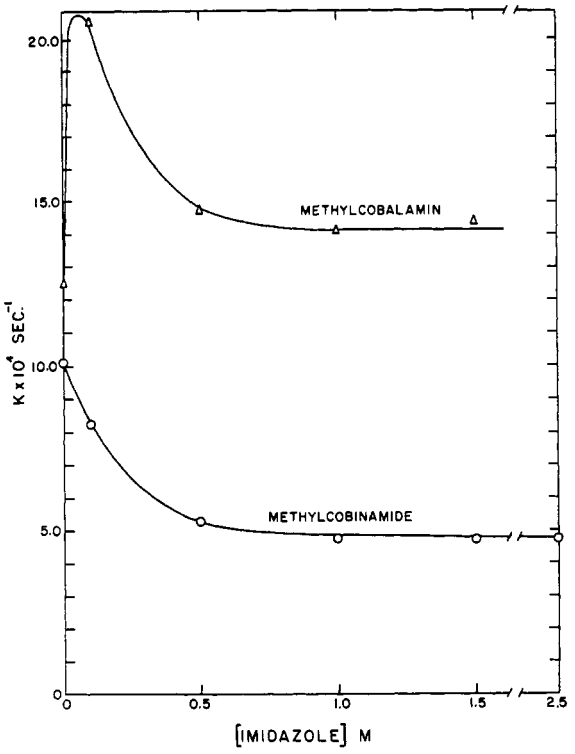

FIGURE 4: Effect of imidazole concentration on the rate of photolysis of methylcobalamin and of methylcobinamide; concentrations of corrinoids and conditions of photolysis were the same as those of the experiments described in Figure 3.

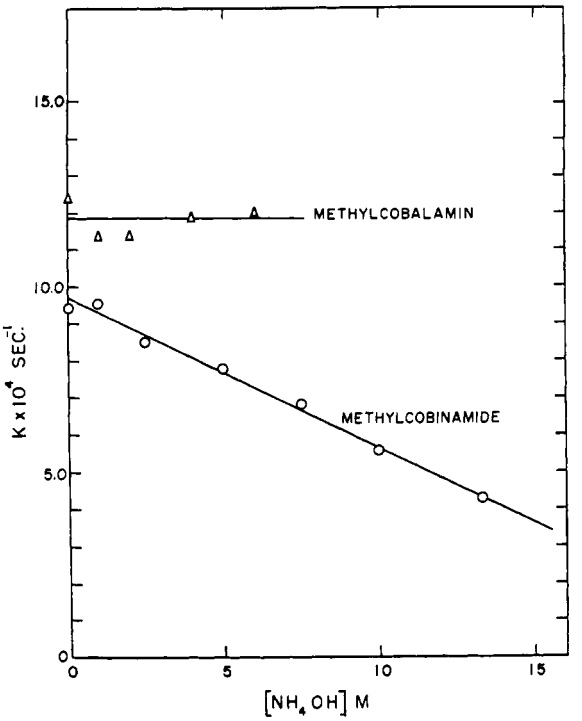

FIGURE 5: Effect of NH_4OH concentration on the rate of photolysis of methylcobalamin and of methylcobinamide. See Figure 3 for experimental detail.

cobalt bond. The effect of cyanide ion on the rate of decomposition of the organometallic bond is shown in Figure 7. Pyridine has a similar but less pronounced effect on the rate of photolysis: in 1.0 M pyridine the rate of photolysis is increased to 15.7×10^{-4} sec^{-1}.

Discussion

The methylcobinamide–ligand complexes serve as model compounds to explain the behavior of methylcobalamin bound to N^5-methyltetrahydrofolate–homocysteine transmethylase. Transmethylase-bound methylcobalamin differs from free methylcobalamin in the photolability of the methyl group linked to cobalt and furthermore, the spectra of holotransmethylase and methylated and propylated holotransmethylase are distinctly different from the spectra of the corresponding free cobalamins. These spectra all show a blue shift of the 525-mμ band (α band) and the spectrum of propylated transmethylase in particular suggests that the 5,6-dimethylbenzimidazole moiety is not bound to cobalt in the enzyme–coenzyme complex. The formation constants of the alkylcobinamide–ligand complexes (Table II) are very small compared with those of the cyanocobinamide–ligand (George *et al.*, 1960) and cobalamin–ligand (Pratt and Thorp, 1966) complexes. For instance, George *et al.* (1960) found the formation constants of cyanoaquocobinamide and dicyanocobinamide to be 10^{14} and 10^{8} M^{-1}, respectively. Similar large differences were found in the pK values for the displacement and protonation of the 5,6-dimethylbenzimidazole moiety in cyanocobalamin (pK_a = 0.1) and in methylcobalamin (pK_a = 2.72) (Hayward *et al.*, 1965). Thus the very small formation constants of the methylcobinamide–ligand complexes reflect the strong nucleophilic character of the methyl group. The difference in formation constants of the methylcobinamide–imidazole and propylcobinamide–imidazole complexes is the result of the positive inductive effect of the extra ethyl group in the latter. A similar difference was observed between methylcobalamin (pK_a = 2.72) and propylcobalamin (pK_a = 3.84), indicating that in the case of propylcobalamin the 5,6-dimethylbenzimidazole ligand is more weakly coordinated to the cobalt atom. In agreement with Firth *et al.* (1967) the data presented in Table II show a direct correlation between the wavelengths of the α band and the formation constant of the complex, suggesting that an increase in electron density on the cobalt atom causes a red shift of the α band. These data also indicate that the formation constants are not determined by the basicity of the ligand. For instance, the very low formation constant of the methylcobinamide–piperidine complex in spite of the high pK_a of the ligand (pK_a = 11.12) is probably due to steric hindrance.

The data presented in Figures 1 and 2 indicate that only the free bases are able to form a coordinate bond with cobalt. The spectra of the methylcobinamide–ligand complexes at low pK values are identical with the spectrum of methylaquocobinamide, suggesting that concomitant with protonation of the ligand is displacement of the ligand by water.

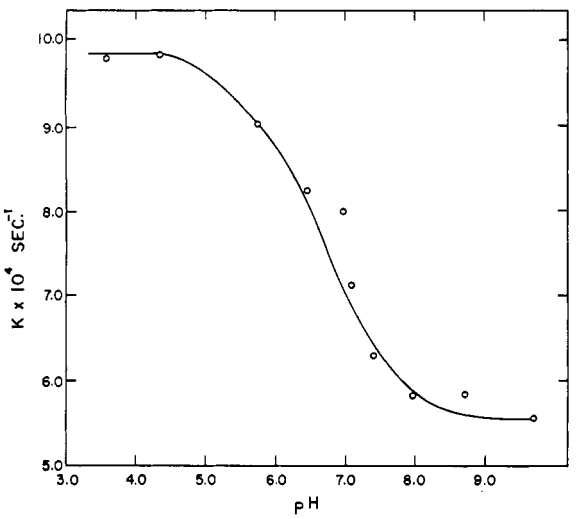

FIGURE 6: Effect of pH on the photolysis of methylcobinamide in the presence of 0.05 M imidazole. See Figures 1 and 3 for experimental detail.

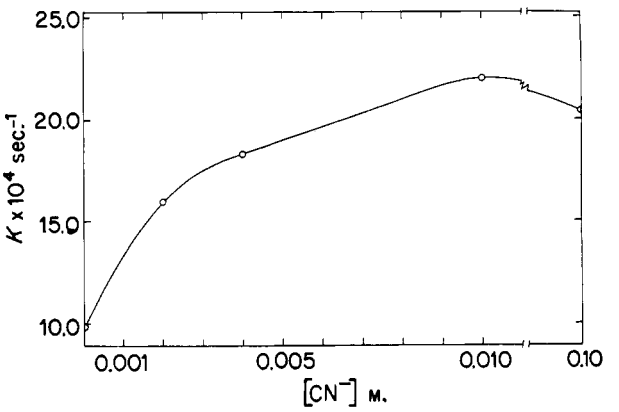

FIGURE 7: Effect of cyanide ion concentration on the rate of photolysis of methylcobinamide. See Figure 3 for experimental detail.

Taylor and Weissbach (1968) have suggested that the increased photolability of methylated holotransmethylase in acid may be related to the displacement of the 5,6-dimethylbenzimidazole moiety from cobalt; however, the data shown in Figure 3 demonstrate that opening of the nucleotide–cobalt coordinate bond renders the carbon–cobalt bond *less* sensitive to light. The pK_a of methylcobalamin calculated from these photolysis data is identical with that determined by the spectral method (Hogenkamp *et al.*, 1965). These data also indicate that, in contrast to our earlier suggestion (Hogenkamp *et al.*, 1965), the homolytic cleavage of the carbon–cobalt bond is influenced by electron donation to or electron withdrawal from the organometallic bond by the ligands on both positions on the cobalt atom (the Y and Z ligands in Table I). Displacement of the 5,6-dimethylbenzimidazole moiety by water in acid solution would make the cobalt atom more electrophilic and as a result the alkyl group would be more tightly held. Similarly any ligand that is able to replace water would decrease the electrophilic character of cobalt and weaken the carbon–cobalt bond. In accordance with these predictions both cyanide ion and pyridine were found to increase the photolability of the organometallic bond of methylcobinamide. Cyanide ion is much more effective than pyridine, because the affinity of cyanide ion for methylcobinamide is much greater than that of pyridine for methylcobinamide (Table II).

In contrast, however, imidazole, 1-methylimidazole, and ammonia were found to decrease the rate of photodecomposition of the carbon–cobalt bond. The stabilizing influence of these three ligands is exactly opposite to the anticipated effect. These results suggest that binding of these ligands to cobalt causes an increase in the electrophilic character of the cobalt atom. Because the formation constant of the methylcobinamide–ammonia complex is much smaller than that of the methylcobinamide–imidazole complex, a much larger concentration of ammonia is required to yield the same photostabilizing effect (compare Figures 4 and 5). Although no explanation can be offered for this effect, the observed stabilizing influence of imidazole and ammonia may explain the unexpected stability of methylated holotransmethylase to light. The following series of reactions could account for the spectral properties of holotransmethylase and for the light stability of methylated holotransmethylase. In holotransmethylase the cobalt–5,6-dimethylbenzimidazole coordinate link is broken and water is coordinated to cobalt; as a result, the spectrum of the holoenzyme is very similar to that of cyanocobalamin in concentrated H_2SO_4 (Hill *et al.*, 1962), with absorbance maxima at 322, 405, and 470 mμ.

In methylated holotransmethylase, the same coordinate bond is broken and a new coordinate bond between a histidine residue of the apoenzyme and cobalt is formed. Although the formation constant for the methylcobinamide–imidazole complex is small, the apoenzyme can provide a local high concentration of histidine by positioning the histidine residue close to the cobalt atom. Analogous to the methylcobinamide–imidazole complex such displacement of benzimidazole by histidine would render the carbon–cobalt bond less susceptible to cleavage by light. As a result of the coordination of histidine the absorbance spectrum of the methylated holotransmethylase would be expected to show a red shift (Table II) and indeed Taylor and Weissbach (1967a,b) found a slight shift in the 470–540-mμ region of the spectrum. When holotransmethylase is propylated the spectrum of the chromophore is altered more drastically. The new absorbance maxima at 370 and 430 mμ and the shoulder at 470 mμ are very similar to those of *n*-propylaquocobinamide, suggesting that although the benzimidazole–cobalt coordinate bond is opened, the histidine residue of the apoenzyme is not able to bind to the cobalt atom. The formation constant of the *n*-propylcobinamide–imidazole complex (Table II) is two orders of magnitude smaller than that of the methylcobinamide–imidazole complex due to the inductive effect of the propyl moiety. This finding suggests that in propylated holotransmethylase the electron-donating effect of the propyl group weakens the electrophilic character of the cobalt atom to such

an extent that the histidine residue is unable to form a coordinate bond. As a consequence the carbon–cobalt bond is not stabilized and is readily cleaved by exposure to light. The decrease in the photolability of the carbon–cobalt bond of methylcobinamide by ammonia suggests that a lysine residue of the apoenzyme may also coordinate its ε-amino function to cobalt and thus stabilize the organometallic bond. However, at physiological pH the amino group of lysine is protonated; the much lower pK_a value of imidazole makes histidine ideally suited for coordination with cobalt.

References

Barker, H. A., Smyth, R. D., and Hogenkamp, H. P. C. (1963), *Biochem. Prepn. 10*, 27.

Ertel, R., Brot, N., Taylor, R., and Weissbach, H. (1968), *Arch. Biochem. Biophys. 126*, 353.

Firth, R. A., Hill, H. A. O., Pratt, J. M., Williams, R. J. P., and Jackson, W. R. (1967), *Biochemistry 6*, 2178.

Friedrich, W., and Bernhauer, K. (1956), *Chem. Ber. 89*, 2507.

George, P., Irvine, D. H., and Glauser, S. C. (1960), *Ann. N. Y. Acad. Sci. 88*, 393.

Guggenheim, E. A. (1926), *Phil. Mag. 2*, 538.

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

Hill, J. A., Pratt, J. M., and Williams, R. J. P. (1962), *J. Theor. Biol. 3*, 423.

Hogenkamp, H. P. C. (1966), *Biochemistry 5*, 417.

Hogenkamp, H. P. C. (1968), *Ann. Rev. Biochem. 37*, 225.

Hogenkamp, H. P. C., and Rush, J. E. (1967), *Biochem. Prepn. 12*, 121.

Hogenkamp, H. P. C., Rush, J. E., and Swenson, C. A. (1965), *J. Biol Chem. 240*, 3641.

Johnson, A. W., Merwyn, L., Shaw, N., and Smith, E. L. (1963), *J. Chem. Soc.*, 4146.

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

Taylor, R. T., and Weissbach, H. (1966), *Federation Proc. 25*, 1649.

Taylor, R. T., and Weissbach, H. (1967a), *Arch. Biochem. Biophys. 119*, 572.

Taylor, R. T., and Weissbach, H. (1967b), *J. Biol. Chem. 242*, 1502.

Taylor, R. T., and Weissbach, H. (1967c), *J. Biol. Chem. 242*, 1509.

Taylor, R. T., and Weissbach, H. (1968), *Arch. Biochem. Biophys. 123*, 109.