On the Reaction of Methylmercuric Hydroxide with Methylcobalamin

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(Z. Naturforsch. **31 c,** 753–755 [1976]; received July 29, 1976)

Methyl Transfer, Kinetic Studies, Corrinoid Coenzymes, Organomercurials

The methylmercury-induced dealkylation of the corrinoid coenzyme methylcobalamin, yielding aquocobalamin and dimethylmercury as products, was studied spectrophotometrically at 350 nm using water as a solvent. Rate data were determined for the pH 7–9 region and also at pH 3.37. Evidence is provided which shows that CH_3Hg^+ serves as the species which accepts the methyl group and also that Hg^{2+} is methylated more rapidly than CH_3Hg^+ is.

The ability of the corrinoid coenzyme methylcobalamin (CH_3-B_{12})* to alkylate mercuric salts, both enzymatically [1, 2] and non-enzymatically [3–10], has attracted considerable attention since this reaction may represent one pathway by which highly toxic methylmercury derivatives are formed in the environment under suitable conditions. Demethylation of CH_3-B_{12} is believed to occur as a simple acid-base catalyzed reaction involving the heterolytic cleavage of the cobalt-carbon σ-bond during the electrophilic attack of Hg(II) (formation of CH_3^-) and yielding, as principal products, methylmercury and aquocobalamin (H_2O-B_{12}) *[,3, 6, 8–10]. While the kinetics and mechanism of this reaction have been investigated in considerable detail by at least two research groups [8–10] very little is known about the continued reaction of methylmercury with the coenzyme. It is reasonable to assume that the monofunctional organomercurial, *viz.*, in the form CH_3Hg^+, can add another CH_3^- carbanion group resulting in the formation of dimethylmercury. The production of dimethylmercury in the presence of methylcobalamin was indeed observed by several workers [1, 2, 5, 6]. However, to our knowledge, no rate data concerning this reaction have ever been published although there seems to be a consensus that Hg^{2+} is methylated more rapidly than CH_3Hg^+ [6, 8].

In this brief communication, we describe the results of experiments in which the kinetics of the reaction

$$CH_3Hg(II) + CH_3-B_{12} \xrightarrow[(H_2O)]{k_1} (CH_3)_2Hg + H_2O-B_{12} \quad (1)$$

were studied spectrophotometrically (Cary/Varian Model 118C spectrophotometer) at 25 °C using water (unbuffered) as solvent. CH_3-B_{12} and H_2O-B_{12} were prepared from vitamin B_{12} (Sigma) according to known procedures [11, 12]. CH_3HgOH was kindly donated by Nor-Am Agricultural Products, Inc. (Woodstock, Illinois). The kinetic studies were executed under pseudo first-order conditions, *i. e.*, in the presence of a large excess of CH_3HgOH, and the reaction rates were evaluated *via* the half-time method. As a rule, reactions were followed for three to four half-times. pH measurements supplemented the kinetic measurements. Further experimental details can be gathered from Fig. 1 and Table I.

Table I. Rate data for the reaction of methylcobalamin [a] with CH_3HgOH in water.

$[CH_3HgOH]$ [mM]	$[CH_3Hg^+]$ [b] [μM]	pH	$t_{1/2} \times 10^{-3}$ [sec]	$k_{1(obs)} \times 10^5$ [c] [sec^{-1}]
4.01	8.57	7.25	26.0	2.67
6.69	8.82	7.46	27.0	2.57
9.36	16.62	7.33	16.0	4.33
10.70	12.57	7.51	20.0	3.47
12.00	12.00	7.58	20.0	3.47
13.40	16.47	7.49	14.5	4.78
26.80	6.73	8.18	35.0	1.98
53.50	9.96	8.31	24.0	2.89
	$\bar{k}_1 = 2.88 \pm 0.15\ M^{-1}\ sec^{-1}$ [d]			

[a] The concentration of CH_3-B_{12} was 77.6 μM.
[b] Evaluated by using the known ionization constant of CH_3HgOH [13].
[c] The reaction was followed at 350 nm and at 25 °C.
[d] Obtained by dividing $k_{1(obs)}$ through $[CH_3Hg^+]$. The value given is the average together with the standard deviation.

In Fig. 1, we have assembled the visible and near-ultraviolet spectra of CH_3-B_{12}, collected both in presence and absence of CH_3HgOH. Curve 1 is the spectrum of CH_3-B_{12} (76 μM) alone. Under the experimental conditions given, the coenzyme is in the so-called "base-on" configuration, *i. e.*, the DMBz * residue is coordinated to the central cobalt atom [3, 7–10]. The dotted curve represents the spectrum of CH_3-B_{12} (76 μM) obtained in presence of CH_3HgOH (7.6 mM) after 46 min of standing while the spectrum given by the dashed curve is

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* The abbreviations and trivial names used are: CH_3-B_{12}, α(5,6-dimethylbenzimidazolyl)-Co-methylcobamide (methylcobalamin); H_2O-B_{12}, α(5,6-dimethylbenzimidazolyl)-Co-aquocobamide (aquocobalamin); vitamin B_{12}, α(5,6-dimethylbenzimidazolyl)-cobamide cyanide (cyanocobalamin); 5,6-dimethylbenzimidazole, "base", DMBz.

that of the same mixture recorded after 250 min of standing. Curve 2 was obtained after the very same sample that gave rise to both the dotted and dashed curves had been standing for more than 12 h. At this point in time, curve 2 changes only insignificantly upon further standing and, moreover, it is identical with the spectrum produced by an authentic sample of H_2O-B_{12}. The spectral alterations are not produced when CH_3-B_{12} is left standing

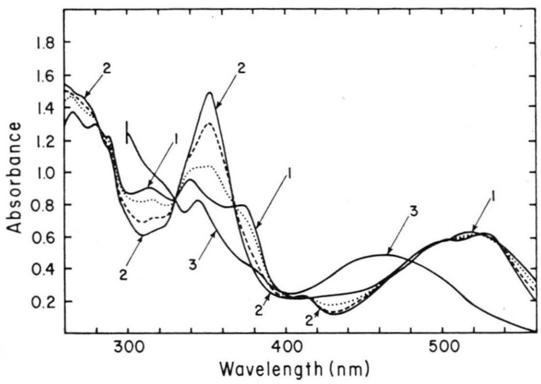

Fig. 1. Changes in the visible and near-ultraviolet spectrum of methylcobalamin (76 μM; curve 1) upon the addition of a hundred-fold excess of methylmercuric hydroxide: dotted curve (the mixture after 46 min of standing); dashed curve (the mixture after 250 min of standing); curve 2 (the mixture after 12 h of standing). Solvent: water; incidental solution pH 7.3. Curve 3: 76 μM methylcobalamin in presence of 9.4 mM CH_3HgOH at pH 3.37 immediately (15 sec) after mixing. The coenzyme is here in the so-called "base-off" form exhibiting the characteristic absorption maximum at 460 nm. Scanning was performed only to 300 nm. Curve 3 will slowly be converted to curve 2 upon standing. For details see text.

in the absence of CH_3HgOH; we conclude therefore that they are indicative of the transmethylation reaction described by Eqn (1) with CH_3-B_{12} in the "base-on" form.

The time course of the reaction between methylcobalamin and CH_3HgOH was followed by monitoring the changes in absorbance at 350 nm. The rate of methyl transfer can obviously be expressed as $d[H_2O-B_{12}]/dt = k_1[CH_3-B_{12}][CH_3Hg(II)]$ which, under pseudo first-order conditions, becomes $d[H_2O-B_{12}]/dt = k_{1(obs)}[CH_3-B_{12}]$. Pertinent data on the rate of the reaction are assembled in Table I. It is readily verified that a log-log plot of $k_{1(obs)}$ *versus* $[CH_3Hg^+]$ yields a straight line with a slope of 0.89. No linear relationship is obtained if log $k_{1(obs)}$ is plotted against $\log[CH_3HgOH]$. We conclude from this that the transmethylation reaction, Eqn (1), is first order with respect to CH_3H^+ and that CH_3Hg^+ is the chemical entity which accepts CH_3^-. This conclusion is also supported by the finding that by decreasing the pH at a given organomercurial concentration, *viz.*, with the help of dilute HNO_3 (nitrate will no complex methylmercury), $k_{1(obs)}$ can be increased. Thus, $k_{1(obs)} = 2.24 \times 10^{-3}\ \text{sec}^{-1}$ when CH_3-B_{12} (76 μM) is incubated with CH_3HgOH (9.4 mM) at pH 3.37. The reason, of course, is that increasing the hydrogen ion concentration also increases the ionization of CH_3HgOH, and CH_3HgOH exists at pH 3.37 to 94% as CH_3Hg^+. On the other hand, the second order rate constant amounts to only $k_1 = 0.254\ \text{M}^{-1}\ \text{sec}^{-1}$ at pH 3.37 and is thus by a factor of about ten smaller than the value found for the pH region 7 – 9 (*cf.*, Table I). The reason here is that at the high methylmercuric cation concentration given CH_3-B_{12} exists predominantly as "base-off" methylcobalamin (*cf.*, curve 3, Fig. 1), *i. e.*, in a configuration where the binding of CH_3Hg^+ to one of the nitrogen binding sites of DMBz has led to the latter's displacement from the central cobalt atom, and it is known that "base-off" methylcobalamin becomes more slowly demethylated than "base-on", methylcobalamin[3, 7–10]. We will address ourselves to the mercury- and hydrogen ion-induced "base-off", "base-on" equilibrium of CH_3-B_{12} more in detail elsewhere[10].

The data presented in Table I show that demethylation of CH_3-B_{12} by CH_3Hg^+ is, in general, a slow process. As to the question whether or not transmethylation proceeds more rapidly when Hg^{2+} is the substrate, no direct answer can be provided for the pH 7 – 9 region since here Hg^{2+} is neither in existence nor remains soluble as $Hg(OH)^+$ and/or $Hg(OH)_2$. However, transmethylation studied at pH values near 3 in the presence of Hg^{2+} and $Hg(OH)^+$ [10] yields a second order rate constant of $k_{(1)} = 3.55 \pm 0.03\ \text{M}^{-1}\ \text{sec}^{-1}$ which would be definitely in support of the generally held belief that Hg^{2+} is methylated more rapidly than CH_3Hg^+ [6, 8].

This investigation has been supported by funds of the University of California and, in part, by Grant No. GM 16282 from the U.S. Public Health Service.

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