rivatives.[38,39] It also explains the success of the reconstitution of Beem et al.[5,37] in which either Cu^{2+} or Ag^{+} is added to apoprotein at pH 3.8 where it binds predominantly at the copper binding site. Our results indicate that the copper binding site is the only strong binding site available at the pH so that competition with a second binding site in the subunit is thereby avoided.

Acknowledgment. Financial support from the NSF and the USPHS (GM 28222) are gratefully acknowledged. J.S.V. is grateful to Professor J. E. Coleman for some helpful comments. In addition we thank P. McDonnell, whose initial observations served as the impetus for the research described here.

Registry No. Superoxide dismutase, 9054-89-1; Zn, 7440-66-6; Cu, 7440-50-8.

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Rates of Cytidine Amino Mercuration by Methylmercury(II) Determined by ^1H NMR Saturation Recovery

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Abstract: Saturation recovery of the amino ^1H resonance is a useful and necessary alternative to line-shape analysis for the determination of rates of cytidine amino mercuration by methylmercury. Reciprocal lifetimes of the free ligand could be obtained by correction of recovery data for dipolar contributions, which were determined from the temperature dependence of recovery in D_2O-H_2O mixtures. Reciprocal lifetimes fall in the range of 2-11 s^{-1} and are influenced by two separate rate processes: At high levels of total CH_3HgOH they are a measure of the forward rate constant of amino mercuration, $k_1 = 1.2 \pm 0.1 \times 10^3$ s^{-1} M^{-1} (free CH_3HgOH), and conform to an activation energy of 17 ± 2 kcal mol^{-1}; at low total [CH_3HgOH], they reflect an additional amino-to-water proton exchange mechanism under conditions associated with significant concentrations of N_3-protonated Cyd (free amino ligand). This latter route is proportional to total cytidine concentration.

Introduction

The interaction of methylmercury with DNA is of interest with respect to both its biological effects and its great value as a tool to study DNA in vitro. Chronic intake of methylmercury at subtoxic levels can produce chromosome damage in humans,[1] presumably through its direct interaction with DNA.[2] In vitro experiments show that methylmercury in millimolar concentrations will melt DNA and at lower concentrations will trap preformed melted DNA segments that are too small to be observed spectrophotometrically.[3,4] The aqueous chemistry of the methylmercury cation, CH_3Hg^{+}, is very similar to that of the proton, which assumes the crucial role in DNA base recognition through hydrogen bonding.[5,6,20]

In anticipation of detailed kinetic studies at the polymer level, recent studies have been undertaken in this laboratory to provide a *kinetic* comparison of methylmercury and the proton in their interactions with mononucleotides. Mixtures of methylmercuric hydroxide and adenosine, adenylic acid, and cytosine provide ^1H NMR spectra characteristic of low rates of mercuration at the amino site.[7,8] ^1H NMR line-shape analysis of separate resonances of the free amino and mercurated amino exchange pair provided mercuration rates in the range of 2-100 s^{-1}. Resonances of the amino protons involved in the mercuration could be studied directly in the case of adenine but not in the cytidine system, owing to the marked frequency dependence of the amino ^1H NMR line shape in Cyd-CH_3HgOH mixtures, which originate from C_4-NH_2 bond rotation.[8]

The present study was undertaken to explore the method of saturation recovery as a direct means to utilize the amino resonances of cytosine for the estimate of mercuration rates. Because of the large (~1 ppm) frequency separation of the cytidine amino proton resonances of the free and bound (-NH_2) forms,[8] saturation recovery by selective presaturation of either resonance should be sensitive to exchange coupling through mercuration and would be initiated by proton transfer from magnetically unsaturated water as the third site. This should provide estimates of mercuration rates independent of the rotamer equilibria that are characteristic of cytosine. In this report it is shown that dipolar contributions determined from the temperature dependence of recovery in D_2O and H_2O are used as corrections to obtain reciprocal lifetimes in agreement with those of the previous study.[8] These reciprocal lifetimes are a measure of amino mercuration and conform to an activation energy of 17 ± 2 kcal mol^{-1}. At low concentrations of methylmercuric hydroxide, recovery is dominated by an additional amino-to-water exchange mechanism proportional to the C(N_3)-protonated form of cytidine.

Experimental Section

Cytidine (Sigma Chemical Co.) was used as supplied. Methylmercuric hydroxide samples were diluted from a 16.8% stock solution kindly provided as a gift from the Agricultural Division of Morton Chemical Co. Methylmercury concentration was determined by the procedure of Libich and Rabenstein[9] with the Varian HA-100 NMR spectrometer of the Department of Chemistry, University of Hawaii. All pH adjustments were made with perchloric acid with the use of Ingold and Beckman pH electrodes as discussed previously.[7] Nominal pH values of 3, 4, and 5 are all within 0.1 pH unit.

All NMR experiments were performed on the Bruker HXS 360 NMR spectrometer at the Stanford Magnetic Resonance Laboratory, Stanford

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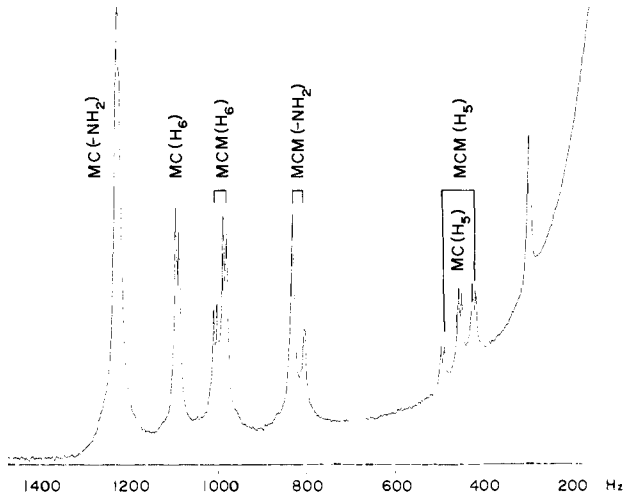


Figure 1. A 360-MHz ^1H NMR spectrum of cytidine in the presence of methylmercuric hydroxide. Long-pulse (Redfield) FT acquisition by quadrature detection was performed on a mixture of cytidine (0.15 M) and methylmercuric hydroxide (0.36 M) at pH 4, 3 °C. Resonance assignments shown were determined by decoupling experiments described previously.[8] Frequency in hertz is relative to H_2O.

University.[10] Saturation transfer experiments were performed with the Redfield pulse sequence[11] employed for quadrature FT acquisition after a time τ following the direction of a saturating pulse at the frequency of one of the exchange-coupled resonances.[12-14] Saturation recovery experiments were repeated to establish that recovery rates were independent of decoupling power and delay times following acquisition. A caveat in studies of this type is to examine recovery data for the additional effect of an overlapping resonance. Preirradiation of a selected rapidly recovering resonance produces partial collapse of an overlapping resonance, whose slower (T_1) recovery will produce a slower continuation of recovery of the selected resonance at long τ values. This produces curvature in first-order plots, which are restored properly to linearity by judicious selection of an infinite time intensity, obtained by correction for recovery of the overlapping resonances. Pulse sequences representing a single recovery time were repeated 20 to 200 times to accumulate data for a single kinetic point. Off-resonance decoupling experiments were performed at all pH conditions for assurance that saturation transfer originated from exchange. Manipulation of digitized data was done with the Nicolet 1180 computer. Recovery rates were obtained by single exponential least-squares analysis of data points fitted with a correlation $r^2 > 0.9$, with most values in excess of 0.95. Recovery measurements for a given sequence of delays between presaturation and acquisition were taken from signal heights of indentically phase-corrected spectra, which were normalized to the infinite time spectrum in terms of all instrumental parameters other than recovery. The first-order description of recovery is expressed as the fraction of saturated protons remaining at time τ by

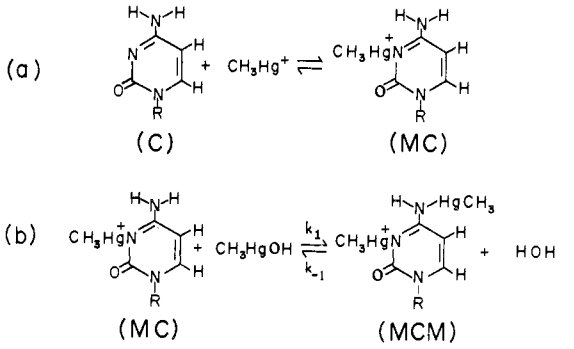
$$\ln (1 - Y_I/Y_\infty) = R$$

where Y_I and Y_∞ are signal height at time τ and at infinite time, respectively, and R is the first-order rate constant for recovery.

Results

Resonance Designation and Assignment. A-360 MHz ^1H NMR spectrum of an aqueous mixture of cytidine and methylmercuric hydroxide (Figure 1) shows resolution of each of the pyrimidine proton resonances (C_5, C_6, and $-NH_2$)[15] into exchange-coupled

Scheme I



pairs. Each resonance of the exchange pair corresponds to the free amino and mercurated amino species, which are designated in Figure 1 as MC and MCM, respectively. All MCM resonances are split further into exchange doublets arising from slow rotation of the mercurated amino group about the C_4–N bond[8] but are considered as a single resonance for purposes of saturation recovery. Each resonance is a coalesced result of three pyrimidine species that interchange through rapid equilibria at the weakly basic endocyclic (N_3) site of Cyd. For example, each of the free amino resonances designated as MC actually represent the free, protonated, and mercurated N_3 species (C, HC, and MC, respectively, where C refers to cytidine, H to the proton, and M to CH_3Hg^+). Abbreviations are defined in ref 15. Similarly, all MCM resonances are coalesced signals of CM, HCM, and MCM, i.e., mercurated amino species that are produced by the same interactions of H and M at the N_3 site described above. The designation of resonances as MC or MCM are made because of the kinetic and molar predominance of these species and the negligible contribution of the other four (C, HC, CM, and HCM) under these conditions.[8] Accordingly the reaction under consideration is shown in Scheme Ib, in which amino mercuration by CH_3HgOH involves species MC, which is produced first by the binding of CH_3Hg^+ to N_3 (Scheme Ia).

Recovery Rates and Exchange Lifetimes. Preirradiation of the MC($-NH_2$) or MCM($-NH_2$) resonances produces partial to complete collapse of both, which is followed by their recovery characterized by 10 to 150 ms half-times. Typical first-order plots of recovery of the MC($-NH_2$) and MCM($-NH_2$) resonances are shown in Figure 2. Recovery of the nondissociable C_6 proton resonance is included to verify the much slower T_1 values typical for this system (insert, Figure 2C). The MC–MCM resonance pair for the C_6 proton represents a simple two-site exchange system, in which

$$R = \frac{1}{T_1} + \frac{1}{\tau_M}$$

where R is the first-order rate constant for recovery and T_1 and τ_M represent times of longitudinal relaxation and amino mercuration, respectively. However, the simultaneous collapse and recovery of MC(C_6) and MCM(C_6) upon saturation of either is produced by resonance overlap at 360 MHz and results in a 1.1 s decay time appropriate for T_1 in this small molecule system. Under these conditions the water T_1 is 2.2 s.

The much faster recovery rates observed for MC($-NH_2$) and MCM($-NH_2$) (Figure 2) reflect a true exchange contribution to recovery in excess of spin–lattice relaxation. The third reaction site, water (Scheme Ib), does not become appreciably saturated by preirradiation of MC or MCM. Thus, water acts as an immediate source of magnetization in MC, primarily through the reverse of amino mercuration (Scheme Ib) and, to a negligible extent, through base-catalyzed amino-to-water exchange (see Discussion). The low zero time values for MC recovery (Figure 2B,D) reflect the fact that preirradiation of MCM($-NH_2$) produces only partial steady-state collapse of MC($-NH_2$). In this case, this fraction of the original intensity of MC($-NH_2$) persists for long MCM($-NH_2$) preirradiation times at higher decoupling power, which verifies true saturation transfer[16,17] through chemical

(10) The Bruker HXS 360 NMR spectrometer is part of a National Facility funded by the National Science Foundation (Grant GR-23633) and the National Institutes of Health (Grant RR-00711).
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(15) Abbreviations used: C, cytosine; Cyd, cytidine; MC, N_3-mercurated cytosine; HC, N_3-protonated cytosine; MCM, MC mercurated at the amino site; HCM, HC mercurated at the amino site; CH_3HgOH_{tot}, total methylmercuric hydroxide; CH_3HgOH, free methylmercuric hydroxide. C_5 and C_6 are carbons 5 and 6 of cytosine and ($-NH_2$) represents the amino resonance, whether it contains two hydrogens or one, e.g., MCM($-NH_2$).

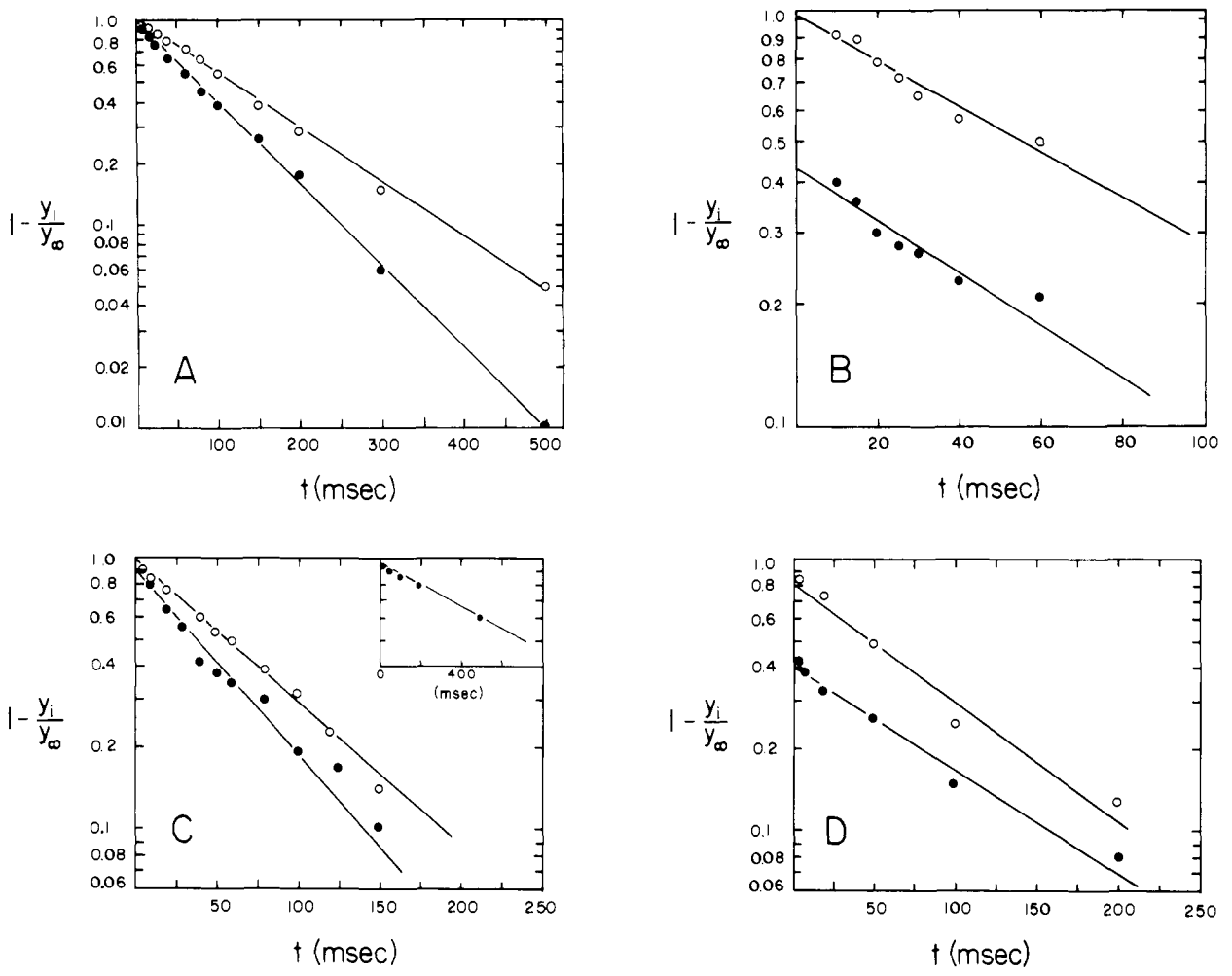

Figure 2. Saturation recovery plots of the amino proton exchange pairs of cytidine in the presence of methylmercury. First-order plots of the fraction of resonance intensity remaining unrecovered vs. time were obtained from mixtures of 0.15 M Cyd and 0.36 M CH_3HgOH_{tot} at pH 3 (A and B) and pH 4 (C and D). Figures A and C are recovery after preirradiation of MC($-NH_2$) and Figures B and D are recovery after selective preirradiation of MCM($-NH_2$) (Figure 1). Probe temperature was 23 °C. The recovery of the overlapping MC and MCM resonances for the C_6 proton at pH 4 is shown in the insert in C. Lines through experimental points are least-squares regression plots with correlation r^2 values in excess of 0.9 obtained from two or three separate experiments: (○) recovery of MCM($-NH_2$); (●) recovery of MC($-NH_2$).

exchange in this system, as opposed to resonance overlap.

Relative contributions of chemical exchange and dipolar relaxation to recovery were obtained from the temperature dependence of recovery rate for D_2O/H_2O solvent mixtures. Arrhenius plots for recovery (Figure 3) are nonlinear and clearly reflect chemical exchange above 23 °C, where recovery is dependent upon temperature, pH, and total CH_3HgOH concentration. At lower temperatures, recovery rates are independent of both temperature and solute composition but exhibit a proportional decrease in 50 and 75% D_2O. Thus, the dipolar correction is obtained from H_2O and D_2O recovery rates at 3 °C treated simultaneously

$$R_{(MC)} \text{ in } H_2O = \frac{1}{T_1} + \frac{1}{\tau_{(MC)}}$$

$$R_{(MC)} \text{ in } 50\%\ D_2O = \frac{0.5}{T_1} + \frac{1}{\tau_{(MC)}}$$

where $R_{(MC)}$ is the recovery rate of MC($-NH_2$), T_1 is the longitudinal relaxation time, and $\tau_{(MC)}$ is the exchange (mercuration) lifetime of the MC resonance. The factor 0.5 reflects the halving of the dipolar contribution in 50% D_2O. Corrections of this type yield, typically, $1/T_1 = 7$–$8\ s^{-1}$ and produce a linear Arrhenius plot of reciprocal exchange lifetimes (Figure 4). It is shown in Figure 4 that reciprocal lifetimes obtained in this manner are in agreement with rates derived from the line-shape of the MC(C_6) resonance according to the method outlined previously.[8] An activation energy of 17 ± 2 kcal mol^{-1} is obtained from regression analysis of the data in Figure 4.

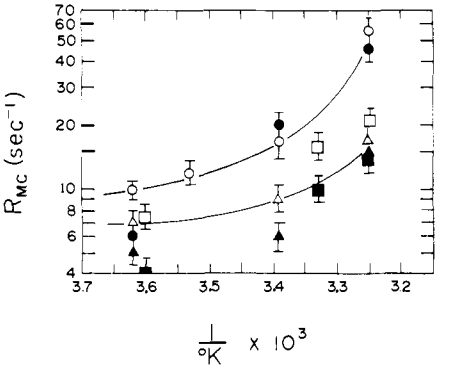

Figure 3. Semilog plot of MC recovery rates vs. reciprocal absolute temperature. Recovery rates determined from first-order magnetization decay slopes in the temperature range 3–35 °C for 0.15 M Cyd under the following conditions: (○) 0.36 M CH_3HgOH, pH 4 in H_2O; (●) 0.36 M CH_3HgOH, pH 4 in 50% D_2O; (△) 0.36 M CH_3HgOH, pH 3 in H_2O; (▲) 0.36 M CH_3HgOH, pH 3 in 50% D_2O; (□) 0.15 M CH_3HgOH, pH 4 in H_2O; (■) 0.15 M CH_3HgOH, pH 4 in 75% D_2O.

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Table I. Mole Fractions of Cytidine Species and Free CH_3HgOH Under Selected Experimental Conditions[a]

[Cyd]/[CH_3HgOH_{tot}]	pH	P_C	P_{MC}	P_{CM}	P_{MCM}	P_{HC}	P_{HCM}	[CH_3HgOH]$_f$, M
0.15/0.36	4	0.0004	0.54	2×10^{-5}	0.23	0.0007	0.23	0.0086
0.15/0.36	3	0.0002	0.65	3×10^{-6}	0.02	0.003	0.28	0.0024
0.15/0.24	4	0.0008	0.6	2×10^{-5}	0.15	0.001	0.26	0.0049
0.15/0.24	3	0.0003	0.67	3×10^{-6}	0.04	0.004	0.28	0.0013
0.15/0.15	4	0.001	0.67	3×10^{-5}	0.01	0.02	0.29	0.0041
0.01/0.1	4	0.0007	0.58	2×10^{-5}	0.16	0.001	0.25	0.0057
0.01/0.1	3	0.0003	0.67	3×10^{-6}	0.04	0.005	0.28	0.0014

[a] Values calculated by iterative root determination of a fourth-order polynomial using the formation constants listed in Table II. APL program is available upon request. Abbreviations are listed in ref 15.

Table II. Log Formation Constants for Methylmercury-Cytidine Equilibria

reaction	log formation constant	ref
(1) $CH_3Hg^+ + OH^- \rightleftarrows CH_3HgOH$	9.41	20
(2) $CH_3Hg^+ + CH_3HgOH \rightleftarrows (CH_3Hg)_2{}^+OH$	2.37	20
(3) $Cyd + H^+ \rightleftarrows CydH^+(N_3)$	4.22	21, 22
(4) $Cyd + CH_3Hg^+ \rightleftarrows CydCH_3Hg^+(N_3)$	4.60	21, 22
(5) $CydCH_3Hg^+(N_3) + CH_3HgOH \rightleftarrows CydH_{-1}(CH_3Hg)_2{}^+(N_3, -NH_2) + H_2O$	1.69	21, 22
(6) $Cyd + CH_3HgOH \rightleftarrows CydH_{-1}CH_3Hg(-NH_2) + H_2O$	0.79	21, 22
(7) $CydH^+(N_3) + CH_3HgOH \rightleftarrows CydH^+(N_3)H_{-1}CH_3Hg(-NH_2) + H_2O$	4.30	8

[a] Reactions taken from ref 21 and 22 were converted to the above form by using a value of 1 for [H_2O] and 10^{-14} for K_W.

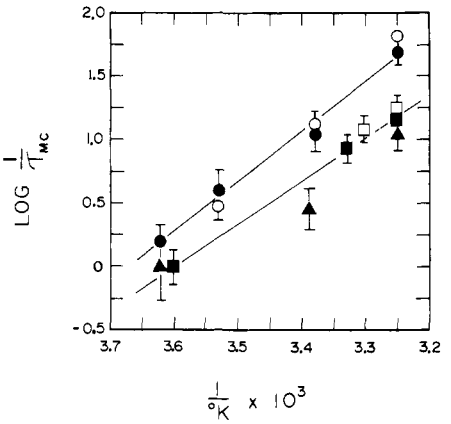

Figure 4. Log_{10} reciprocal lifetimes of MC ($1/\tau_{MC}$) vs. reciprocal of absolute temperature. Reciprocal lifetimes were obtained from recovery rates corrected for dipolar contributions as described in the text for 0.15 M cytidine solutions (H_2O) containing CH_3HgOH at 0.36 M, pH 4 (●); at 0.36 M, pH 3 (▲);and at 0.15 M, pH 4 (■). Open figures are reciprocal lifetimes obtained from line-width measurements on the C_6 proton resonance (MC) (Figure 1).

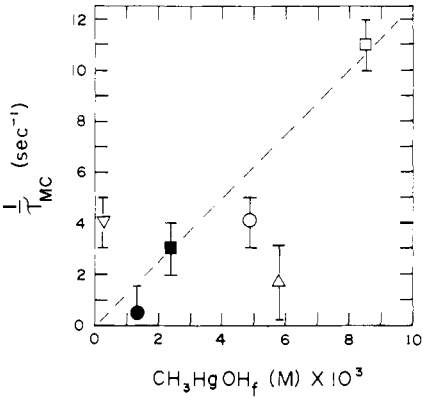

Figure 5. Reciprocal lifetimes of MC resonance vs. free CH_3HgOH. Reciprocal lifetimes were obtained by correction of the recovery rates at 23 °C described in the text. Concentrations of CH_3HgOH_f were calculated by iterative root extraction of a polynomial derived from a total of seven species (ref 7): (▽) 0.15 M ($CH_3HgOH)_{tot}$; (○) 0.24 M ($CH_3HgOH)_{tot}$; (□) 0.36 M ($CH_3HgOH)_{tot}$; all of which correspond to 0.15 M Cyd. (△) 0.1 M ($CH_3HgOH)_{tot}$, 0.01 M Cyd. Closed and open figures correspond to pH 3 and 4, respectively.

Reciprocal Lifetimes and Mercuration Rates. The dependence of MC($-NH_2$) recovery rates on total CH_3HgOH seen in Figure 1 is translated to the terms of Scheme Ib by a plot of recovery rate as a function of the concentration of free methylmercury hydroxide, CH_3HgOH_f. All points corresponding to the condition [CH_3HgOH_{tot}] > 0.25 M conform to a linear relationship within experimental error, whose slope provides a forward rate constant, $k_1 = 1.2 \times 1 \times 10^3$ s^{-1} M^{-1} (CH_3HgOH_f). It is apparent that two experimental conditions are associated with a large deviation from the linear relationship. At 0.15 M CH_3HgOH_{tot}, the reciprocal lifetime is several times higher than predicted rates for this concentration, while rates corresponding to 0.1 M Cyd and 0.1 M CH_3HgOH_{tot} show a marked deviation downward. An examination of species concentrations under these conditions reveals a correlation between these high and low deviations and the concentration of HC, the N_3-protonated free amino ligand. The data of Table I, calculated from the formation constants listed in Table II, show that the mole fraction of HC is quite low for 0.24 and 0.36 M CH_3HgOH_{tot} (<0.004) but increases by an order of magnitude (0.02) under the high-rate condition, [CH_3HgOH_{tot}] = 0.15 M. When the total Cyd concentrations are lowered to 0.01 M with the same approximate amount of CH_3HgOH_{tot} (0.1 M), both the HCl concentration and the reciprocal lifetime are conspicuously low. As a result, the data for [CH_3HgOH_{tot}] > 0.24 M provide rate constants for mercuration. From the forward constant, k_1, presented above, the reverse rate constant of Scheme 1b is calculated as

$$\frac{1}{\tau_{(MCM)}} = k_{-1} = k_1\left(\frac{P_{MC}}{P_{MCM}}\right)[CH_3HgOH_f] = 25\ s^{-1}$$

taken from the pH 4, 0.36 M CH_3HgOH data of Table I and [H_2O] = 1 M. Recovery rates, dipolar contributions, and reciprocal lifetimes are listed in Table III for all conditions in H_2O.

Discussion

The z magnetization (M_z) of the amino protons of MC at any time after the saturation pulse will be a function conforming to the reaction event, in which the single amino proton that is replaced by CH_3Hg has two available sites, MC or H_2O (Scheme Ib). Recovery will follow the relation

$$\frac{dM_z^{MC}}{dt} = -M_z^{MC}R_{MC} + M_z^{H_2O}R_{H_2O}$$

where M_z^{MC} and $M_z^{H_2O}$ are the z magnetizations (at time τ) of

Table III. Amino Proton Resonance Recovery Rates and Reciprocal Lifetimes For Cytidine-Methylmercury Mixtures at 23 °C[a]

[Cyd]/[CH_3HgOH]$_{tot}$	pH (±0.05)	R_{MC}, s^{-1}	R_{MCM}, s^{-1}	$1/T_1^{MC}$, s^{-1}	$1/\tau_{MC}$, s^{-1}	obsd P_{MC}	obsd P_{MCM}	$1/\tau_{MCM}$,[b] s^{-1}
0.15/0.36	3	9 ± 1	6 ± 1	7 ± 1	2 ± 1	0.71	0.29	14 ± 2
0.15/0.24	3	7 ± 1	5 ± 1	6 ± 1	≤1	0.79	0.21	≤6
0.01/0.1	3	6 ± 1		5 ± 1	≤1	0.87	0.13	≤14
0.15/0.36	4	17 ± 2	9 ± 1	7 ± 1	11 ± 1	0.5	0.5	22 ± 2
0.15/0.24	4	11 ± 1	11 ± 1	7 ± 1	4 ± 1	0.7	0.3	18 ± 2
0.15/0.15[c]	4	11 ± 1		7 ± 1	4 ± 1	~0.9	~0.1	70 ± 10
0.01/0.1	4	9 ± 1		7 ± 1	2 ± 1	0.7	0.3	10 ± 1

[a] All recoveries were measured after preirradiation of the MC(-NH_2) resonance (Figure 1). Similar values were obtained in all cases after MCM preirradiation. [b] Calculated from $1/\tau_{MCM} = P_{MC}/\tau_{MC}P_{MCM}$. [c] Rate values obtained by interpolation between 3 and 27 °C data (Figure 4). MCM resonance not visible.

the proton at the MC and H_2O sites, and R_{MC} and R_{H_2O} are their respective recovery rates. Because water protons are not saturated magnetically, the second term is constant, which reduces recovery of the MC resonance to a simple first-order description dependent on its lifetime and magnetization,

$$\ln \frac{M_z^{MC}}{M_0^{MC}} = -R_{MC}\tau$$

where M_0^{MC} is the recovery limit magnetization of MC(-NH_2), and τ is the time between saturation and FT pulse acquisition. Recovery is measured conveniently by signal lights (Y), rather than areas, and is expressed in the form $\ln (1 - Y_1/Y_\infty) = R_{MC}$, where Y_1 and Y_∞ are signal intensities at time τ and infinite time, respectively. According to foregoing results the overall recovery rate is described by three terms:

$$R_{MC} = \frac{1}{T_1^{MC}} + \frac{1}{\tau_{(CH_3HgOH_f)}} + \frac{1}{\tau_{HC}}$$

where $1/T_1^{MC}$ is the dipolar contribution obtained from the 3 °C recoveries, $1/\tau_{(CH_3HgOH_f)}$ is amino-to-water exchange according to Scheme 1b, and $1/\tau_{HC}$ is the additional and variable exchange, which is significant only under the condition [CH_3HgOH_{tot}] < 0.24 M and [Cyd] ≥ 0.15 M. At higher [CH_3HgOH_{tot}], the concentration of HC is too low to produce a measurable contribution and the reciprocal lifetime becomes

$$\frac{1}{\tau_{MC}} \simeq \frac{1}{\tau_{(CH_3HgOH_f)}} = k_1[CH_3HgOH_f]$$

where k_1 is the second-order forward rate constant for Scheme Ib. That the source of the $1/\tau_{HC}$ contribution is derived from the N_3-protonated form of the free ligand is based on previous experience, aside from the circumstantial correlation with [HC] under appropriate conditions (Table I). In the absence of methylmercury, the most significant exchange route for NH_2-to-H_2O exchange has been shown to proceed through the N_3-protonated intermediate.[18,19] At pH 4, self-catalysis of exchange is maximal, reflecting the role of $C(N_3)$ as the amino proton acceptor from HC, and is dependent upon total cytidine concentration at the concentrations used in this work.[18]

Because reciprocal lifetimes have been expressed as low integral values, it is not possible to obtain accurate estimates of $1/\tau_{MCM}$ from the relation

$$\frac{2P_{MC}}{\tau_{MC}} = \frac{P_{MCM}}{\tau_{MCM}}$$

where P refers to the relative mole fractions of the two species, and the factor 2 accounts for the two-proton area of the MC(-NH_2) resonance. The values of $1/\tau_{MCM}$ obtained in this way are greater than the observed recovery rates of the MCM resonance, which are usually somewhat lower than R_{MC} (Figure 2, Table III). The low values of R_{MCM} can be accounted for by the implication of Scheme Ib, which provides no direct route for magnetization of MCM from water. The recovery of MC would be rate limiting for the magnetization of MC, i.e.,

$$R_{MCM} = \frac{R_{MC}(1/\tau_{MCM})}{R_{MC} + (1/\tau_{MCM})} + \frac{1}{T_1^{MCM}}$$

This relation accounts for $R_{MCM} \leq R_{MC}$ for values of $1/T_1^{MCM}$ of 0.5–1 s.

A question remains regarding the use of a two-proton area as appropriate for the estimate of $1/\tau_{MCM}$. It is possible that exchange arising from C_4–NH_2 bond rotation could be rate limiting for amino mercuration. It has been shown in previous studies that the separate amino protons of the free (N_3) cytosine ligand differ by an order of magnitude with respect to their reactivity in proton transfer reactions.[18,19] A persistence in this reactivity difference in the N_3-mercurated species is expected in the N_3-mercurated form (MC), owing to the very small inductive effect of the N_3-bound CH_3Hg^+ on the amino acidity.[7,8] As seen in Figure 1, the rotamer populations are unequal, occurring in the ratio of about 3 to 1. Furthermore, the appearance of a single -NH_2 resonance [MC(-NH_2) in Figure 1] is not a result of coalescence by rapid rotation but results from a virtual superposition of the separate resonance chemical-shift responses to $C(N_3)$ mercuration, indicating very slow rotation at lower temperature.[8,19] It is possible, therefore, that recovery of MC measures chiefly the exchange mercuration of *one* of the two rotamers and that the values of $1/\tau_{MCM}$ are approximately half the values listed in Table II. Resolution of this question would require accurate recovery measurements of the rotamer and nondissociable proton resonances at field frequencies greater than 360 MHz for sufficient elimination of exchange-paired resonance overlap.

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