INTERACTION OF N-METHYL PHENAZINIUM RADICAL CATION WITH SINGLE-STRANDED POLYRIBOADENYLIC ACID

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SUMMARY: N-Methyl phenazinium radical cation ($MPH^{\dot{+}}$) is known to interact with DNA in a manner analogous to an acridine dye (1). In the present communication, we have investigated the interaction of $MPH^{\dot{+}}$ with single stranded polyriboadenylic acid (poly A) through the combined use of electron paramagnetic resonance (EPR) and Fourier-transform proton magnetic resonance (FT-PMR). Use of a paramagnetic dye enables us to obtain average distances between three protons of poly A nucleotide and the unpaired electron on the dye, giving direct evidence for the π-π complexation of the dye with the adenine base. In addition, kinetic parameters for the complexation reaction are evaluated.

Materials and Methods

$MPH^{\dot{+}}$ radical was synthesized as perchlorate crystals from phenazine methosulfate (obtained from Sigma) according to the method developed earlier (2). Poly A (Sigma) (m.w. ~900,000 from viscosity measurement) was dialyzed against 0.1 M NaCl prior to use. Unbuffered D_2O solution of poly A (65 mM) which contained 4 mM EDTA and 0.1 M sodium ion (pH = 6.0 as pH meter reading) was used for PMR measurement. The pH for EPR measurement was maintained at 6.0 by a sodium acetate buffer (0.05 M). Both measurements were carried under deaerated conditions.

Pulse FT-PMR measurements were made with a JEOL PS-100 spectrometer equipped with a FT unit (PFT-100) operating at 100 MHz. EPR measurements utilized JEOL PE-3X.

Results and Discussion

Figure 1 shows the central portion of the EPR spectra of $MPH^{\dot{+}}$ in the presence (a) and the absence (b) of poly A taken at 25°C. It is

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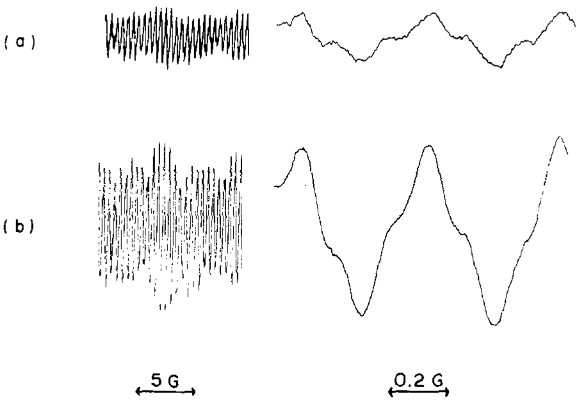

Figure 1. High resolution EPR spectrum of $MPH^{\dot{+}}$ radical in the presence (a) and the absence (b) of poly A. pH 6.0 by 0.05 M acetate buffer. $[MPH^{\dot{+}}]$ = 0.2 mM, [poly A phosphate] = 1.0 mM, 25°C. A central part of the spectrum is expanded in the spectra shown on the right.

clear from the comparison of the two that the unbound species of $MPH^{\dot{+}}$ giving the high resolution spectrum, is decreased by the addition of poly A. This indicates that the EPR spectrum of $MPH^{\dot{+}}$ bound by poly A is too broad to be observed under high resolution conditions. Such a behavior has previously been observed with $MPH^{\dot{+}}$ complexed by double-stranded DNA (1). Assuming a simple bimolecular association

$$MPH^{\dot{+}} + \text{poly A} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} MPH^{\dot{+}}\text{- poly A complex} \qquad (1)$$

we obtain $\sim 10^{-3}$ M (in monomer unit) as an approximate value for K_D $(= k_{-1}/k_1)$. Here K_D is approximately given by $\frac{I \cdot [\text{poly A}]}{(I_0 - I)}$ where I_0 and I are the peak heights of the high resolution EPR spectra in the absence and presence of poly A.

In this treatment, we have assumed that the width of the high resolution spectrum of the unbound radical is unchanged in the presence of the polymer. The expanded spectrum shown on the right of each high resolution spectrum indeed shows that no appreciable broadening occurs in the "unbound" spectrum. This finding also tells us that the rate of reaction of eq. (1) is slow enough that it does not affect the line width of the narrowest line (width ~100 mG) observed. Namely, the

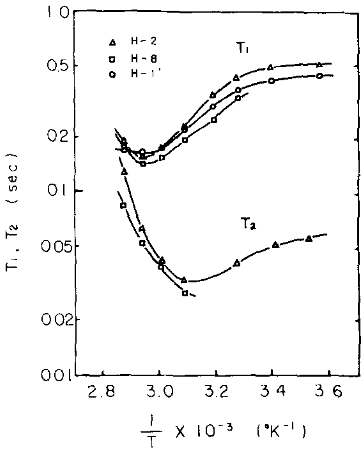

Figure 2. Temperature dependence of T_1 and T_2 for protons of poly A complexed with $MPH^{+\cdot}$ radicals. pH 6.0, [sodium ion] = 0.1 M, [poly A phosphate] = 65 mM, [$MPH^{+\cdot}$] = 0.07 mM.

life time of the unbound radical must be longer than the longest T_2 of the "unbound" spectrum, i.e. 10^{-6} sec. In the present situation where $[MPH^{+\cdot}]_{bound} \simeq [MPH^{+\cdot}]_{unbound}$, the life time ($\tau_b$) of the bound radical should also be nearly equal to the life time of the unbound radical. It is concluded, therefore, that $\tau_b > 10^{-6}$ sec or $k_{-1} < 10^6$ sec^{-1}.

Figure 2 shows temperature dependence of the longitudinal relaxation time (T_1) and the transverse relaxation time (T_2) for three protons in the polymer, H-2, H-8, and H-1' in the presence of $MPH^{+\cdot}$ ($[MPH^{+\cdot}]/[\text{poly A phosphate}] \sim 1 \times 10^{-3}$). Longitudinal relaxation times of these protons are markedly shortened by the presence of $MPH^{+\cdot}$. For example at 67°C in the absence of $MPH^{+\cdot}$ T_1 for H-2, H-8, and H-1' is 1.45, 0.51, and 0.65 sec respectively. It is clear that T_1 is determined dominantly by $MPH^{+\cdot}$-nucleotide complex. The magnitude of K_D indicates that most of $MPH^{+\cdot}$ is bound under these conditions, and the fraction of poly-nucleotide in the bound state is 10^{-3}.

In the present situation where the bound fraction (b) of the poly A nucleotide is small ($b \ll 1$), observed T_1 is related to τ_b, T_{1b} (T_1 in the $MPH^{+\cdot}$-complexed nucleotide), and T_{1o} (T_1 in the absence of $MPH^{+\cdot}$) in the following manner (3) :

$$1/T_1 = b/(T_{1b} + \tau_b) + 1/T_{10} \qquad (2).$$

Similarly for T_2,

$$1/T_2 = b/(T_{2b} + \tau_b) + 1/T_{20} \qquad (3).$$

The observed decrease of T_2 with temperature up to 50°C indicates that in this temperature range, the relaxation is dominated by τ_b, which at 25°C is equal to 6.0×10^{-5} sec from Fig. 2 and the value of T_{20} (0.20 sec). This value of τ_b is consistent with the absence of broadening of the EPR spectrum. Combination of the value $k_{-1}=\tau_b^{-1}= 1.7 \times 10^4$ sec^{-1} with the estimate of K_D from the EPR measurements gives the value $k_1 \sim 2 \times 10^7$ M^{-1} sec^{-1} for the rate of association at 25°C .

T_{1b} is entirely determined by the dipolar interaction with the unpaired electron of MPH$^{\dot{+}}$ separated by distance r from the proton in question through the relation

$$1/T_{1b} = (3/10)(\gamma_e^2\gamma_H^2\hbar^2/r^6)(\tau_c/(1 + \omega_H^2\tau_c^2)) \qquad (4)$$

where γ_e and γ_H are the gyromagnetic ratio for electron and proton, respectively and $\hbar$ is the Planck's constant divided by 2π. T_1 goes through a minimum at ~67°C, indicating that, at this temperature, the rotational correlation time (τ_c) of the complex is given by $\omega_H\tau_c \sim 1$ or $\tau_c \sim 1.6 \times 10^{-9}$ sec, where ω_H is the Larmor frequency of a proton. We then obtain reciprocal 6th power-averaged distances of H-2, H-8, and H-1' of the nucleotide in the complex to the unpaired electron to be 4.7, 4.8, and 4.9 Å, respectively. These distances are in close agreement with the case of 5'-AMP complexed with MPH$^{\dot{+}}$ (4), and are those expected for a π-π complex between MPH$^{\dot{+}}$ and adenine moiety with approximate separation of the base and dye planes of 3.8 Å. This result is one of the few direct evidences in support of a π-π complexation of a dye with a polynucleotide base in solution.

A more detailed structure of the complex of MPH$^{\dot{+}}$ and poly A will result from measurement of the relaxation times of the polynucleotide ^{13}C in the complex and a more accurate determination of the distribution of electron spin density in the radical. Such studies are now in progress.

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