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Structure of a DNA Intercalation Complex as Determined by NMR Using a Paramagnetic Probe

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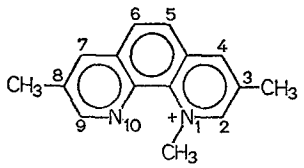
Abstract. Longitudinal relaxation rates of the protons of the 3,8-dimethyl-N-methyl-phenanthrolinium (DMP) cation in solutions containing DNA are strongly affected by the addition of the paramagnetic manganese (II) ions due to the electron-nuclear dipolar interaction in the ternary Mn-DNA-DMP complex. Two possible models for the DMP-DNA intercalation complex are examined and one of them is unequivocally discriminated on the basis of the proton relaxation data. It is concluded that in the intercalation complex the long axis of the DMP molecule is almost perpendicular to the hydrogen bonds of the DNA base-pairs.

Key words: DNA — Intercalation — NMR — Paramagnetic Probes.

Introduction

The insertion of an aromatic ring between the base-pairs of DNA, known in the art as intercalation, is of major importance in governing the interaction between small molecules (*e.g.* drugs), and possibly aromatic residues of proteins, and nucleic acids (Lerman, 1961; Gabbay *et al.*, 1973a and references therein). Several classes of aromatic derivatives of organic cations have been employed in order to probe the detailed nature of the intercalation process. Particularly useful towards this end seem to be the series of N-methyl phenanthrolinium cations. These planar cations have been found to intercalate between DNA base-pairs as evidenced by the severe broadening of the proton magnetic resonance signals, the enhanced viscosity, the induced circular dichroism, and by the dramatic stabilization of the helix resulting from the cation-DNA interaction (Gabbay *et al.*, 1973b). Recent electron paramagnetic resonance (EPR) studies of the binding of manganese (II) to DNA have shown that conditions can be achieved such that the phenanthrolinium cation and Mn^{2+} are simultaneously bound to DNA (Reuben and Gabbay, 1975). Thus a mapping study of the intercalation complex using the paramagnetic effects of Mn^{2+} on the proton relaxation times of the organic cation became feasible. Paramagnetic ions cause a large enhancement in the relaxation rates of nuclei in their vicinity through the electron-nuclear hyperfine coupling. The main mechanism of this effect is dipolar interaction, the magnitude of which is proportional to the inverse sixth power of the electron-nuclear distance. Thus nuclear relaxation rates may be used in distance determinations.

Presented in this communication are the manganese effected longitudinal relaxation rates of the protons of the 3,8-dimethyl-N-methyl phenanthrolinium (DMP) cation in the presence of DNA. From the estimated manganese-proton distances it is possible to inequivocally discriminate between two possible modes of intercalation of this compound.



3,8-dimethyl-*N*-methyl phenanthrolinium (DMP)

Theoretical Background

The theory of the NMR relaxation method using paramagnetic probes is well documented (Mildvan and Cohn, 1970; Cohn and Reuben, 1971; Dwek, 1973). For easy reference we briefly summarize some of the main points and the formulae used in the present work.

The increment in the relaxation rate of a given nucleus due to the presence of a paramagnetic ion is given by

$$1/T_{1p} = P_M/(\tau_M + T_{1M}), \tag{1}$$

where P_M is the molar fraction of the nuclei in question in the vicinity of the paramagnetic ion, where their mean residence time and the relaxation time are τ_M and T_{1M}, respectively. If there is no spin delocalization from the paramagnetic ion to the ligand, only the electron-nuclear dipolar interaction will determine the magnitude of $1/T_{1M}$, which in this case is given by

$$1/T_{1M} = D\, r^{-6} f(\tau_c), \tag{2}$$

where D is a known constant for given nucleus and electronic spin (for protons and Mn^{2+}, $D = 1.424 \times 10^{-31}$ cm^6/sec^2), r is the distance between the two, and for macromolecular complexes

$$f(\tau_c) = 6\,\tau_c/(1 + \omega_I^2 \tau_c^2), \tag{3}$$

where ω_I is the nuclear resonance frequency, and τ_c is the correlation time modulating the dipolar interaction. The shortest among the rotational correlation time, τ_I, the mean residence time, τ_M, and the electron spin relaxation time, T_{1e}, will dominate the correlation time since

$$1/\tau_c = 1/\tau_r + 1/\tau_M + 1/T_{1e}. \tag{4}$$

Materials and Methods

The synthesis of DMP was carried out as previously described (Gabbay *et al.*, 1973a). The binding isotherm for the DMP-DNA complex was determined by equilibrium dialysis according to previously published methods (Gabbay *et al.*, 1973a). The dialysis was carried out at 8° in a plexiglass block using 0.6 mM DNA-phosphate, 0.15 to 0.30 mM DMP, 1 mM $MnCl_2$, 25 mM NaCl, and 10 mM 2-(N-morpholino)-ethanesulfonic acid buffer at pH 6.2. Equilibration was allowed to proceed for 43 hrs and the concentration of free DMP (in the DNA free compart-

Table 1. Spectral assignments, manganese effected relaxation rates, and manganese-proton distances for the DMP-DNA intercalation complex

Proton	δ, ppm[a]	$1/T_{1P}$, sec^{-1}	r, Å	r_i/r_1
1	3.68	11.14	3.03	[1.00]
2	7.65	2.29	3.94	1.30
3	1.409	< 0.1	> 6.5	> 2.14
4	7.35	7.92	3.21	1.06
5	6.44	17.34	2.81	0.926
6	6.35			
7	6.70	10.66	3.05	1.01
8	1.264	< 0.1	> 6.5	> 2.14
9	7.53	8.28	3.18	1.05

[a] Downfilled from tert-butanol.

ment) was then determined spectrophotometrically from the absorption at 317 nm ($\varepsilon = 5960$) using a Gilford 240 spectrometer.

Proton magnetic resonance (PMR) spectra were recorded on the Varian HR-220 Spectrometer (equipped with a Fourier transform accessory) of the high-frequency NMR facility located at the Johnson Research Foundation, University of Pennsylvania, School of Medicine. Longitudinal relaxation times were measured by the 90°-homogeneity spoil-90° pulse sequency of McDonald and Leigh (1973). The samples were made in D_2O and contained 45 mM DMP, 23 mM NaCl, 0.2 mM DNA-phosphate, and a small amount (ca. 5 mM) of tert-butanol, the methyl signal of which served as an internal reference. Small aliquots of $MnCl_2$ solutions were successively added up to a total final concentration of 11 mM. The measurements were carried out at the ambient probe temperature of 17°.

Results

The PMR spectrum of DMP consists of 9 absorptions, 2 of which appear as an AB quartet corresponding to protons at positions 5 and 6, the other being singlets. The spectral assignment was based on the relative deshielding effects of the positive charge and of the nitrogen at position 10 upon the relative chemical shifts, and on a comparison between the spectra of a series of phenanthrolinium cations with different substitutions (Gabbay *et al.*, 1973 b). The chemical shift assignments are given in Table 1. Addition of 0.2 mM DNA-phosphate to 45 mM DMP in the presence of 23 mM NaCl had a negligible effect on both the chemical shifts and the relaxation rates. Subsequent addition of $MnCl_2$ causes the coalescence of the AB quartet into a single line and therefore no distinction could be made between protons 5 and 6. The longitudinal relaxation rates (corrected for non-specific paramagnetic effects in the bulk by subtracting the relaxation rate of the tert-butyl protons) increase (titrate) with increasing Mn^{2+} concentration until a limiting value is approached, indicating saturation of the manganese sites on DNA. The relaxation rate values used in the calculations are those obtained at the final $MnCl_2$ concentration of 11 mM. They are listed in the third column of Table 1. In the absence of DNA this concentration of $MnCl_2$ had no measurable effects on the relaxation rates.

In order to determine the fraction of DMP cations bound to DNA, a series of equilibrium dialysis experiments was carried out under conditions related to those of the NMR experiments. From a Scatchard-plot analysis of the results it was found that the maximum number of intercalating sites per base (phosphate) is 0.12, *i.e.* on the average 4.2 base-pairs form an intercalating site, with an association constant of 9.12×10^3 M. Thus, at the concentration employed for the NMR experiments, the fraction of bound DMP is estimated to be $P_M = 5.33 \times 10^{-4}$.

The markedly different relaxation rates for the different protons suggest that conditions of rapid exchange are attained, *i.e.*, that $\tau_M \ll T_{1M}$. An upper limit for τ_M is obtained from the largest value of $1/T_{1p}$ listed in Table 1, since $\tau_M + T_{1M} = P_M T_{1p}$ and $\tau_M < P_M T_{1p}$. Thus one obtains $\tau_M < 3.1 \times 10^{-5}$ sec. The rotational correlation time for the DNA molecule in this preparation can be shown to be very long, of the order of 10^{-4} (*cf.*, *e.g.*, Reuben *et al.*, 1975, and references cited therein). The most likely correlation time modulating the dipolar interaction seems to be the electron spin relaxation time, as has been found to be the case in many other macromolecular complexes (*cf.*, *e.g.*, Dwek, 1973). Its value was estimated from the EPR spectral data on the manganese-DNA (Reuben and Gabbay, 1975) and the manganese-nucleotide (Reed *et al.*, 1971) complexes using the approximation (Rubinstein *et al.*, 1971) of Bloembergen and Morgan (1961), and found to be $T_{1e} = 2.8 \times 10^{-8}$ sec at the magnetic field employed in this work. One now has a good estimate of all the parameters required in order to use Eq. (2) for the calculation of manganese-proton distances. The values obtained are listed in the fourth column of Table 1. Because of the sixth root dependence of the distance upon the other parameters in Eq. (2), its value is subject to much smaller uncertainties than those of the other factors. In this regard it should be emphasized that the relative distances for the different protons listed in the last column of Table 1 are particularly accurate, since they are free of the many assumptions and depend solely on the sixth root of the observed increments of the relaxation rates.

Discussion

In principle the intercalation of the phenanthrolinium cation between the base-pairs of DNA can result in two major structures: one, in which the long molecular axis is almost perpendicular to the hydrogen bonds of the DNA base-pairs, and another in which the axis is almost parallel to the hydrogen bonds. These two possibilities are depicted in Fig. 1, (a) and (b), respectively. It should be pointed out that parallel and perpendicular geometries for the intercalation of proflavine to DNA have been postulated respectively by Lerman (1961) and by Blake and Peacocke (1968). However, recent attempts to resolve the orientation of the intercalated dye molecule have been unsuccessful (Müller *et al.*, 1973).

A variety of physical and spectral data, including the binding parameters of Mn^{2+} and other cations to DNA, the EPR and CD spectra of the manganese-DNA complex, and the NMR spectra of manganese-nucleotide complexes, have led to the conclusion that there is an appreciable coordination of Mn^{2+} to the base nitrogens (N-7) of DNA (Reuben and Gabbay, 1975, and references cited therein). With the manganese ions located at the base nitrogens, it is clear that in the structure shown in Fig. 1 (a) the methyl group at the phenanthrolinium nitrogen is much closer to a Mn^{2+} ion than the methyls at position 3 and 8 of the phenan-

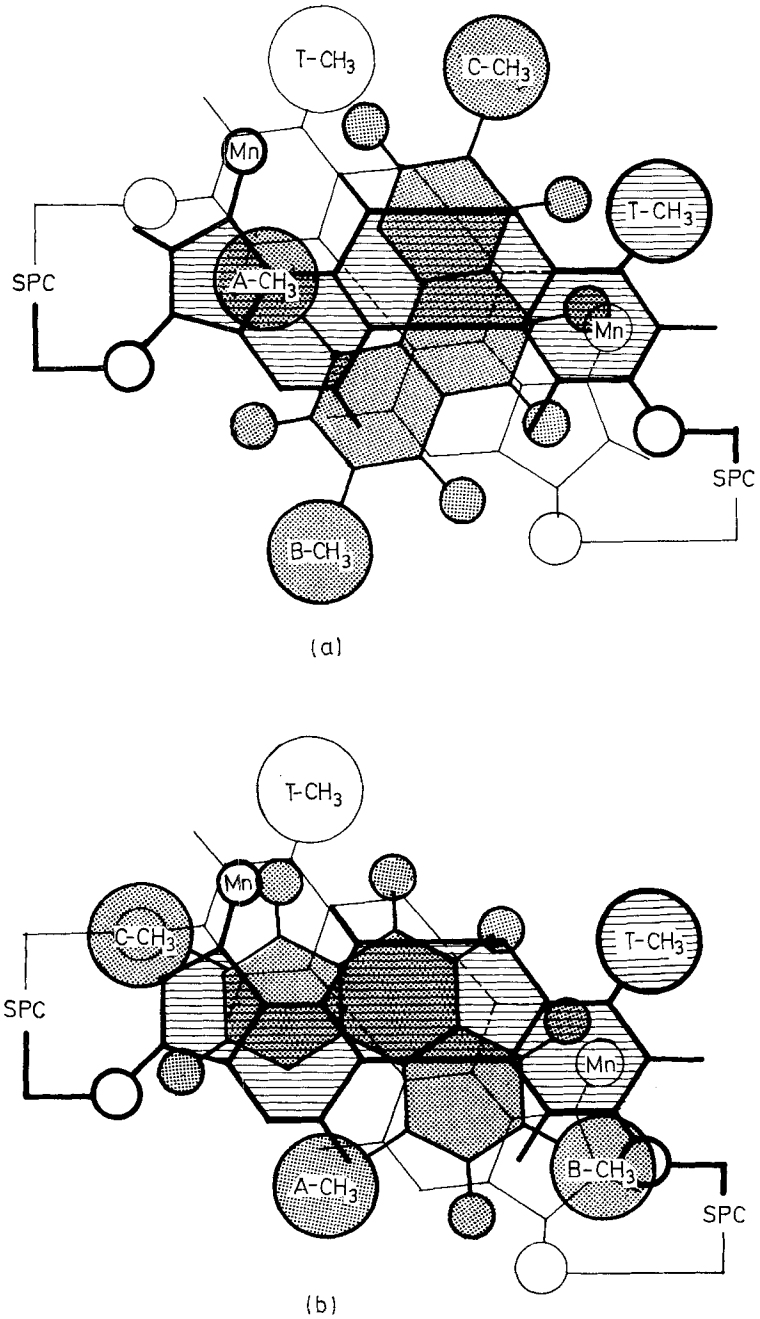


Fig. 1. Schematic representation of the two possible structures of the DMP-DNA intercalation complex. View is along the DNA axis. The base-pair on the top is shown by the heavier lines and its axis is lined. The dotted area represents the DMP molecule. Methyl groups labeled A, B, and C correspond respectively to those at positions 1, 3 and 8 of the phenanthrolinium ring. The following distances (in Å ± 0.3 Å) of the methyl groups were measured on the models from Mn_l, Mn_r, P_l, and P_r, respectively, where the subscripts l and r refer to the right and left halves of the structures, respectively. Structure (a): A — 4.5, 7.8, 7.0, 11.5; B — 8.0, 9.0, 9.3, 9.5; C — 6.8, 6.2, 12.2, 11.0. Structure (b): A — 6.5, 7.0, 8.0, 10.0; B — 10.0, 4.5, 14.0, 5.0; C — 3.6, 10.0, 5.0, 15.0

throlinium ring, and also the protons at ring positions 5 and 6 are closer to a Mn^{2+} ion than those at 4 and 7. These observations as well as similar comparisons for the other protons are in perfect agreement with the relative distances obtained from the relaxation rates (*cf.* Table 1). On the other hand, the relation of the manganese-proton distances in the structure shown in Fig. 1 (b) is almost diammetrically opposite to the distances listed in Table 1. It is thus clear that the NMR results can unequivocally discriminate between the two possible modes of intercalation of the DMP cation. Thus the plausible structure of the DMP-DNA intercalation complex is one in which the long molecular axis is almost perpendicular to the hydrogen bonds of the DNA base-pairs. This conclusion is in agreement with previous physical and spectral data for the same system (Gabbay *et al.*, 1973b).

Some of the absolute distances resulting from the interpretation of the proton relaxation rates are somewhat shorter than might be anticipated from the minimum distance of 3.4 Å required for the intercalation of an aromatic ring between DNA base-pairs. This apparent discrepancy results from the neglect of the possible dipolar interaction of the protons with manganese ions located on the phosphate groups. However, as may be seen from Fig. 1, location of Mn^{2+} ions on the phosphate groups will result in the same sequence of relative manganese-proton distances as that with the Mn^{2+} located at the base nitrogens.

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