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^{31}P NMR and Viscometric Studies of the Interaction of Meso-Tetra(4-N-methylpyridyl)porphine and its Ni(II) and Zn(II) Derivatives with DNA

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ABSTRACT. The interactions of meso-tetra(4-N-methylpyridyl) porphine (TMPyP) and its Zn(II) and Ni(II) derivatives with DNA have been investigated by ^{31}P NMR and viscometric titrations. TMPyP and its Ni derivative increase the viscosity of linear DNA, cause unwinding and reverse coiling of superhelical DNA, and induce a separate downfield peak in the ^{31}P NMR spectrum of DNA. The Zn derivative slightly decreases the viscosity of linear DNA, does not unwind superhelical DNA, and does not give a downfield NMR peak. The main DNA ^{31}P NMR signal is shifted slightly upfield on either the addition of TMPyP or the Ni compound. These results indicate that TMPyP and the Ni(II), but not the Zn(II), derivative bind to DNA by intercalation.

INTRODUCTION. Metal ions which interact with DNA range from simple cations which help maintain nucleic acid structure in vivo (1) to base binding species such as the platinum antitumor agents (2,3). We have found that ^{31}P NMR is an excellent method for analysis of conformational effects involved in the binding of organic intercalators (4,5), simple metal species (3), and base binding metal compounds (3,6) to DNA. The ^{31}P NMR signal of DNA shifts downfield on addition of all intercalators (3,5) as predicted by the empirical and theoretical analysis of phosphate chemical shifts by Gorenstein and co-workers (reviewed in 7). The binding kinetics of actinomycin with DNA are much slower than with other intercalators (8). Addition of actinomycin to DNA results in a spectrum with a separate downfield signal and a ^{31}P peak near that for uncomplexed DNA. Reaction of DNA with the platinum antitumor agent cis-$[Pt(NH_3)_2Cl_2]$ gave a downfield ^{31}P NMR signal while the trans isomer treated in the same manner gave a single peak near that for unreacted DNA (3,6). Simple metal species such as the alkaline earths cause either upfield or no shifts in the DNA ^{31}P NMR signal (3).

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Water soluble porphyrin compounds are extremely interesting model systems for use in evaluating porphyrin biological effects. It has been proposed that meso-tetra(4-N-methylpyridyl)porphine (TMPyP), shown in Figure 1, binds to DNA by intercalation (9,10). Metal containing derivatives of this porphyrin can also be prepared and absorption and C.D. spectral results have suggested that the Ni(II) derivative can bind to DNA by intercalation while the Zn(II) derivative does not intercalate (10). As part of our binding and structural analysis of metal species-DNA interactions, we report here the effects of TMPyP and its Zn(II) and Ni(II) derivatives on DNA solution viscosity and ^{31}P NMR parameters.

Materials and Methods. The porphyrin derivatives used here were supplied by Drs. R.F. Pasternack and E.J. Gibbs. The preparation and properties of these compounds have been reported (10). DNA samples were sonicated to approximately 200 base pairs in length and prepared for ^{31}P NMR as previously described (4). For NMR experiments DNA samples were lyophilized from aqueous buffer and redissolved in an equivalent amount of 99.8% D_2O containing 0.01% trimethylphosphate as an internal standard. Concentrations of aqueous prophyrin stock solutions were determined using extinction coefficients (10), appropriate quantities of the solutions were lyophilized, and the porphyrin dissolved directly into the DNA solution in D_2O buffer. Spectra were accumulated with quadrature detection on either a JEOL FX60Q (24.15 MHz for ^{31}P) or an IBM WP-200SY (81.01 MHz for ^{31}P) NMR spectrometer at 0.01 M DNA phosphate in 10 mm NMR tubes. Typically 8-10,000 scans at 24 MHz or 2-4,000 scans at 81 MHz were obtained with fast Fourier transformation of 8K time domain points, a 90° pulse, five T_1 delay time, and 0.5-1 Hz (24 MHz) or 3-4 Hz (81 MHz) line broadening. Broad band proton decoupling at 24 MHz or power-gated decoupling (two watts between acquisition and four watts during acquisition) at 81 MHz was used for proton decoupled spectra. Solvent deuterium was used for field frequency lock. Temperature measurements were made using the ^{31}P chemical shift thermometer suggested by Gorenstein and co-workers (11) under the same instrumental conditions as collection of spectra. Viscometric titrations were conducted with 600 base pair sonicated calf thymus DNA or closed circular superhelical Col E_1 DNA with electronic timing at 30°C as previously described (12).

RESULTS. Viscometric titrations of sonicated DNA with TMPyP and its Ni(II) and Zn(II) derivatives are shown in Figure 1. TMPyP and Ni(II)-TMPyP both increase the viscosity of DNA while the Zn(II) derivative slightly decreases the viscosity. Viscometric titrations of closed circular superhelical Col E_1 DNA with TMPyP and the Ni(II) derivative gave the characteristic unwinding and reverse coiling maxima curves which are typical of intercalators (12) while the Zn(II) compound gave no evidence for unwinding. Viscometric titrations of DNA (especially linear DNA) were extremely difficult to carry out. Porphyrin

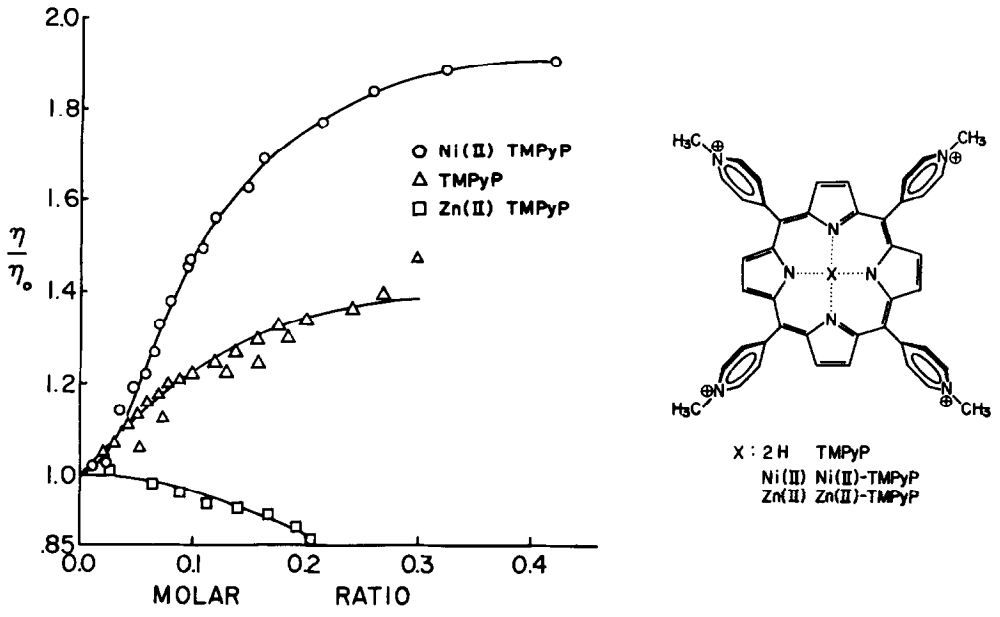

Figure 1. Viscometric titrations of sonicated calf thymus DNA with TMPyP (Δ), and its Ni(II) (O) and Zn(II) (□) derivatives are shown. Experiments were conducted at 30°C in PIPES buffer (0.01 M piperazine-N'N'-bis[2-ethanesulfonic acid], 0.001 M EDTA, 0.1 M NaCl, pH 7.0) using Ubbelohde viscometers. The ratio of reduced specific viscosity of the DNA-porphyrine complex (η)to the reduced specific viscosity of pure DNA (η_0) is plotted as a function of the molar ratio of porphyrine derivative to DNA phosphate.

derivatives had to be added in increments of about 20 μl from dilute solution (approximately 10^{-4} M) with constant stirring to DNA in a viscometer. Salt concentrations had to be at least 0.1 M at 30°C or slow time dependent changes in viscosity and very erratic readings were observed.

In Figure 2 ^{31}P NMR spectra of DNA and TMPyP-DNA complexes at several molar ratios of porphyrin to phosphate are shown. These spectra are quite different from those obtained with most intercalators (3-4) and most closely resemble the spectra obtained on adding actinomycin to DNA (4). A broad downfield peak or peaks is especially apparent in the spectrum at the highest molar ratio. The large DNA peak centered near 4.3 PPM broadens on addition of TMPyP and shifts upfield or in the opposite direction of the shifts obtained with ethidium bromide and other simple intercalators. No other intercalators have been found to cause upfield shifts of a DNA ^{31}P NMR peak. Essentially identical ^{31}P spectra were obtained at 81 MHz with linewidths in hertz being approximately three times greater than at 24 MHz.

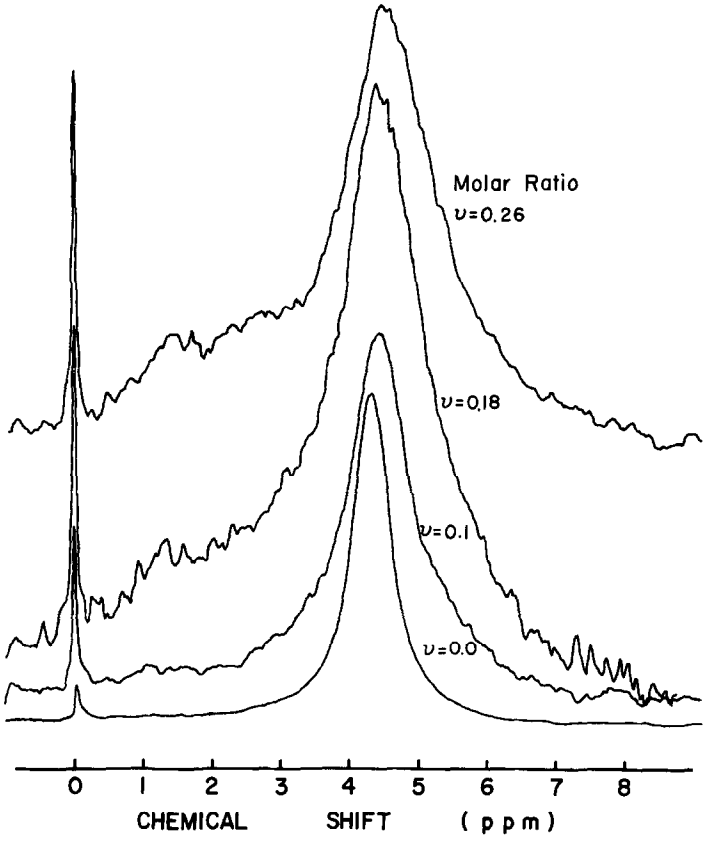

Figure 2. ^{31}P NMR spectra for approximately 200 base pair sonicated DNA (bottom spectrum) in the presence of varying ratios of TMPyP are shown. The reference at 0 PPM is trimethylphosphate and DNA peaks are upfield from this reference. Experiments were conducted at 24 MHz at 30°C in a D_2O solution of the buffer described in Figure 1. Other conditions are described in the Methods section.

In Figure 3 the results of heating a DNA-TMPyP complex (at a molar ratio of porphyrin to phosphate of approximately 0.2) are shown. The linewidths of both the upfield and downfield peaks begin to decrease as the temperature is increased, and the downfield peak begins to shift significantly upfield while the large upfield peak shifts slightly downfield. By 80°C the two peaks have essentially become one fairly sharp peak at 4.1-4.2 PPM.

Spectra for the Ni(II) derivatives of TMPyP with DNA are quite similar to those obtained with TMPyP itself (upfield shifted main peak with downfield shoulder). With the Zn(II) derivative on the other hand the main peak shifts slightly upfield but the downfield peak, characteristic of intercalation, is not present.

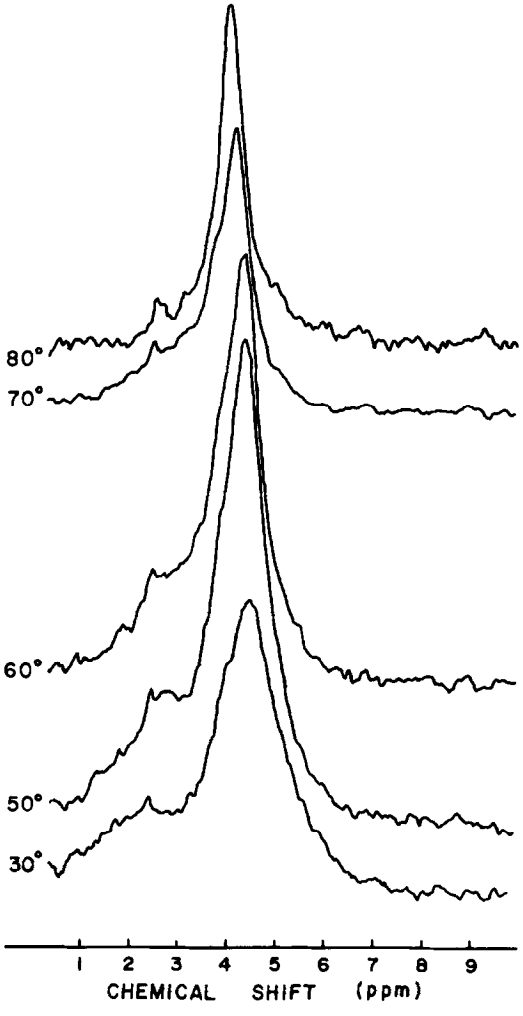

Figure 3. ^{31}P NMR spectra at 24 MHz are shown as a function of temperature for a TMPyP-DNA sample at a molar ratio of 0.25 moles of porphyrine to DNA phosphate. Samples were prepared and spectra collected as described in Figure 2 and the Methods section.

DISCUSSION. We have previously shown that the DNA complex of many intercalators gives a single resolvable ^{31}P NMR peak which is downfield from uncomplexed DNA (4). Actinomycin D, which has unusually slow binding kinetics with DNA (8), is an exception to this behavior and gives a separate downfield peak or peaks in ^{31}P spectra of DNA (4). As can be seen in Figure 2, TMPyP behaves in a manner quite similar to actinomycin. This agrees with the viscosity results of Figure 1 and with previous studies that suggest that TMPyP binds to DNA by intercalation (9,10). The multiple peaks in the ^{31}P spectra also

indicate that TMPyP shows site selectivity and/or slow exchange among DNA binding sites. The upfield shift of the DNA phosphate signal in Figure 2 is also quite interesting. With Actinomycin D, the upfield peak shifted by at most 0.02 PPM from uncomplexed DNA (7) and this is close to the experimental error. With TMPyP, however, the upfield shift of this peak from uncomplexed DNA (4.30 PPM) is approximately 0.2 PPM. This shift could represent an effect due to the high charge density of intercalated TMPyP influencing the chemical shift of neighboring phosphate groups, to a perturbed conformation of DNA, or it could be due to outside bound TMPyP causing upfield shifts of DNA phosphate signals as seen, for example, with tetralysine (7), Mg^{++}, and Ca^{++} ions (3). We are investigating these questions in more detail. It should be noted that these shifts suggest that the conformational distortions induced in the sugar phosphate chains of DNA by the prophyrin derivatives are extremely localized and, in fact, can be separated into effects at and away from intercalation sites. ^{31}P NMR is probably the only method for determining the specific short and long range effects of unwinding agents on the double helix.

When the TMPyP complex (molar ratio=0.25) is heated above 60°C, the two signals of Figure 2 essentially collapse into a single ^{31}P peak (Figure 3). This could be due to denaturation of the complex with release of TMPyP or due to approach of the system to the fast exchange limit. Since spectrophotometric Tm measurements at 260nm suggest that the DNA-TMPyP complex is stable to over 100°C, we do not feel that the NMR results can be explained by DNA denaturation. Variable temperature studies at several field strengths and with synthetic double helical polymers are now in progress to investigate these points in more detail.

With both viscometric (Figure 1) and ^{31}P NMR results, the Ni(II) compound behaves in a manner quite similar to metal free TMPyP. These results suggest that Ni(II)-TMPyP also intercalates between base pairs of DNA. Both the viscometric and NMR techniques, on the other hand, indicate that under these conditions, the Zn(II)-TMPyP derivative does not bind to DNA, to any significant extent, by intercalation. These results for the Ni(II) and Zn(II) de-

rivatives agree with rapid kinetic, absorption and C.D. spectral studies on the interaction mechanisms of these compounds with DNA (10).

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