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Preferential Interaction of Manganous Ions with the Guanine Moiety in Nucleosides, Dinucleoside Monophosphates, and Deoxyribonucleic Acid*

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ABSTRACT: Manganous ions were found to decrease the melting temperatures of several DNAs as the concentration of Mn^{2+} was increased from a Mn^{2+}:DNA-P molar ratio of 3.7 to 15.5. The destabilizing effect of the metal ion increased as the (G + C) content of the DNA increased. When Mn^{2+} was present during the melting of DNA, the hypochromic spectra were altered and these changes were more apparent in DNAs with 50% or greater (G + C) content. These results suggest that the metal ion interacts with the base moieties of the DNA molecule, apparently with some preference for sites on guanine and/or cytosine. Nuclear magnetic resonance (nmr) spectra of the dinucleoside monophosphates TpT, ApA, GpA, ApG, and CpA reveal that the Mn^{2+} possesses a very marked preference for association with guanine over the other three bases. The interaction of Mn^{2+} with nucleosides was then studied by nmr spectroscopy. The results again showed a marked preference for Mn^{2+} association with the guanine ring, providing a good indication that the dinucleoside monophosphate results reflected the existence of a discrete metal ion binding site on the guanine ring.

Divalent cations are essential components of many enzymatic reactions involving DNA. Most such reactions can proceed in the presence of either Mg^{2+} or Mn^{2+}, but changes in the kinetics and specificity of some of these reactions have been described when Mn^{2+} is substituted for Mg^{2+}. Some of the effects of Mn^{2+} subsitution on the reactions catalyzed by RNA polymerase (2.7.7.6) from *Eschericha coli* and DNase I (3.1.4.5) are as follows. When Mn^{2+} was substituted for Mg^{2+}, the rate of *in vitro* RNA synthesis catalyzed by RNA polymerase increased when DNA from calf thymus or various bacterial sources was used as the template but decreased when DNA from several bacteriophages was used as the template (Furth *et al.*, 1962). Substitution of Mn^{2+} for Mg^{2+} in this reaction also was found to lead to a decrease in the rate and yield of chain initiation by ATP (Maitra *et al.*, 1967) and an increase in the amount of enzyme bound to native DNA (Sternberger and Stevens, 1966). The rate of hydrolysis of DNA by DNase I increased upon Mn^{2+} substitution for Mg^{2+} (Wiberg, 1958), and the mechanism of hydrolysis has been found to change from single-chain scission to double-chain scission (Melgar and Goldthwait, 1968). Further, the presence of Mn^{2+} allows DNase I hydrolysis of the poly(dC) chain of dI:dC polymer which does not occur in the presence of Mg^{2+} (Bollum, 1965). The substitution of Mn^{2+} for Mg^{2+} also stimulates the rate and extent of DNA synthesis catalyzed by *Micrococcus luteus* DNA polymerase when either *M. luteus* DNA (72% G + C) or poly(dG:dC) is used as template while with DNAs or polymers of high (A + T) content no differences in cation effect are observed (Litman, 1971).

These results, as well as others not discussed here, suggest that Mn^{2+} may alter the interaction between DNA and the enzyme proteins. Eichhorn and Shin (1968) have presented data indicating that while Mg^{2+} binds primarily to the phosphate groups of DNA, Mn^{2+} interacts with the base rings as well as with the phosphate groups. The work presented here describes a further investigation of the interaction of Mn^{2+} with DNA. Two types of experiments were employed. The first type concerned the effect of Mn^{2+} on the denaturation and hypochromic spectra of DNAs of varying base composition; the second type utilized proton nuclear magnetic resonance (nmr) spectroscopy to examine the interaction of Mn^{2+} with nucleosides and dinucleoside monophosphates in order to identify the site of metal ion complex formation with the bases. The results of the first type of study indicate that Mn^{2+}

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interacts preferentially with the G·C base pairs in the DNA molecule. The nmr studies then reveal that this is probably due to specific metal ion binding at a site on the guanine ring.

Materials

DNA was extracted from *M. lysodeikticus* and *Proteus mirabilis* by the method of Oishi (1968) and further purified by ethanol precipitation and treatment with trypsin, RNase, Pronase (500 μg/ml, 3 hr at 37°), and deoxycholate by a modification of the method described by Young and Spizizen (1961). The DNA was then precipitated with isopropyl alcohol as described by Marmur (1961) and the enzyme–deoxycholate treatments were repeated. After the final ethanol precipitation, the DNA was dissolved in 0.01 M Tris (pH 7.4). Sufficient 1.0 M Tris (pH 7.4) was added to make the final concentration of Tris (0.1 M). This solution was dialyzed for 5 hr against 10 volumes of 0.1 M Tris (pH 7.4)–0.01 M EDTA, and then overnight against 10 volumes of 0.1 M Tris (pH 7.4). A few drops of chloroform were added to the final DNA solutions, which were stored at 4°.

E. coli K 12 DNA, obtained from General Biochemicals, was dissolved in 0.1 M Tris (pH 7.4) and dialyzed as described above. The alternating copolymer of adenine and thymine (d(A-T)) was purchased from Biopolymers Inc., in a solution of 0.01 M Tris (pH 7.9).

The concentrations of DNA and d(A-T) in the final solusolutions were determined by the diphenylamine reaction (Burton, 1956).

Adenosine, guanosine, deoxycytidine, deoxyguanosine, deoxyinosine, and thymidine, were purchased from Calbiochem as the "A grade" compounds. Deoxyadenosine was obtained from Nutritional Biochemicals, cytidine from P-L Biochemicals, and D_2O (99.77%) from Columbia Organic Chemicals, Inc. Adenylyl-(3′–5′)-guanosine (ApG), guanylyl-(3′–5′)-adenosine (GpA), thymidylyl-(3′–5′)-thymidine (TpT), and cytidylyl-(3′–5′)-adenosine (CpA) were obtained from Sigma Chemical Co. Adenylyl-(3′–5′)-adenosine (ApA) was obtained from Calbiochem. All chemicals were reagent grade unless otherwise stated. All solutions were prepared with glass-distilled, deionized water.

Methods

Melting Temperature Determinations. Stock solutions of DNA were diluted to a final concentration of 21–23 μg/ml in 0.01 M Tris (pH 7.4) containing varying concentrations of $MnCl_2$ or $MgCl_2$. Solutions were kept at room temperature for at least 1 hr before the start of the experiment. The increase in uv absorption with increasing temperature was recorded with a Gilford automatic spectrophotometer equipped with a thermosensor. A temperature programmer attached to the water bath was adjusted so that the temperature increased at a rate of 1.5°/min. Readings were corrected for thermal expansion using the coefficient of expansion of water. Orginally, absorbances of the DNA solutions were compared to blanks containing buffer plus appropriate amounts of $MnCl_2$. When it was found that the addition of $MnCl_2$ to buffer solutions did not alter the absorbance in the concentrations used, all solutions were read against blanks containing only buffer.

The melting temperature, T_m, is defined as the temperature at which one-half of the total observed increase in absorbance at 260 nm is reached. Some samples showed a biphasic increase in absorbance, in which the second increase occurred with increasing temperature. In these instances, three T_m's were calculated; the first T_m is defined as that temperature at which one-half the change in absorbance at the initial slope occurred, the second T_m is that temperature at which one-half the increase at the second slope occurred, and the total T_m is that temperature at which one-half of the total change in absorbance occurred. The initial slope of the melting curve was quite steep in these samples and the difference between total T_m and the first T_m was within the average deviation of the measurements.

Hypochromic Spectra Determinations. Two absorption spectra from 220 to 290 nm at 5-nm intervals were measured for each sample that was used for the melting temperature curves. The first spectrum was taken at room temperature; the second at the temperature at which the absorbance at 260 nm had reached its maximum. The samples were held at the high temperature for at least 5 min before the spectra were read to allow temperature equilibration. The high-temperature readings were corrected for thermal expansion using the expansion coefficient of water. Corrections were also made for changes in solvent absorbance with temperature. The hypochromic spectrum is the difference between the two spectra (Felsenfeld and Hirschman, 1965) and was calculated for each sample, where $\Delta A = A\lambda$ (high temperature) $- A\lambda$ (room temperature) in which $A\lambda$ is absorbance at wavelength, λ.

Nuclear Magnetic Resonance Studies. Nmr spectra were obtained using either a Varian A-60A or a Varian HA-100 spectrometer. Solutions of nucleosides were 0.05 and 0.1 M as determined by weight, and the pH of these solutions was adjusted to 6.8 ± 0.3. Varying amounts of a 0.01 M solution of $MnCl_2$ were added to the nucleoside solutions. Measurements were made at 80° in order to ensure complete solution of the nucleosides. Solutions were generally made in D_2O. Early experiments in which deoxyguanosine was dissolved in D_2O were difficult to interpret because the proton at C-8 exchanged fairly rapidly with the solvent at the high temperatures used.

Nmr spectra of the dinucleoside monophosphates were run at magnet temperature (30°). A capillary tube containing tetramethylsilane was used as the external lock. The dinucleoside monophosphates were dissolved in D_2O and the pH of the solutions was adjusted to pH 7.0 ± 0.2. Molarity of the samples were determined from their absorbance at 260 nm, correcting for the hypochromicity of the dinucleoside monophosphates as reported by Toal *et al.* (1968). The molarity of TpT was calculated from the weight of the dissolved sample.

Results

Melting Temperature Determinations. The effects of Mn^{2+} and Mg^{2+} on the melting temperature (T_m) of various DNA solutions and of d(A-T) are shown in Table I. At a ratio of metal ion to DNA-P of 3.7–3.8 the T_m values are all higher than in the absence of metal ions, but the values with Mn^{2+} are consistently lower with a given DNA than with Mg^{2+} present. As the concentration of Mn^{2+} was increased further, the T_m's of the three DNA samples decreased and, furthermore, this decrease was greater as the (G + C) content of the DNA increased. In the case of *M. lysodeikticus*, the T_m's in the higher concentrations of $MnCl_2$ occur at temperatures lower than in 0.01 M Tris alone. The T_m of d(A-T) did not decrease as the concentration of Mn^{2+} was increased although it was always somewhat lower than in equivalent amounts of Mg^{2+}. In the presence of $MgCl_2$, the T_m's of both d(A-T) and *E. coli* DNA increased with increasing cation concentration.

Precipitation of denatured DNA was observed at Mn^{2+} concentrations of 3 mM and higher. Eisinger *et al.* (1963) re-

TABLE I: Melting Temperatures of Several DNA Samples in 0.01 M Tris (pH 7.4), Plus Various Concentrations of $MnCl_2$ or $MgCl_2$.[a]

DNA Source and Concn of DNA-P	$MnCl_2$ ($\times 10^4$ M)	$MgCl_2$ ($\times 10^4$ M)	°C			
			T_m	Second T_m	Total T_m	ΔT_m
E. coli						
6.5×10^{-5} M	0	0	69.5 (4) ± 0.4			
50% G·C[b]	2.5	0	80.4 (2) ± 0.1			10.9
	5.0	0	78.9 (2) ± 0.1	84.2 ± 0.6	79.0 (2) ± 0.2	9.5
	7.5	0	76.7 (1)	83.3	77.0 (1)	7.5
	10.0	0	74.2 (1)	80.8	74.7 (1)	5.2
	0	2.5	85.2 (1)			15.7
	0	10.0	87.8 (1)			18.3
M. lysodeikticus						
6.8×10^{-5} M	0	0	79.6 (3) ± 0.3			
72% G·C[b]	2.5	0	84.4 (2) ± 0.6			4.8
	5.0	0	80.6 (2) ± 0.6			1.0
	7.5	0	79.0 (2) ± 0	84.4 ± 0.8	79.1 (2) ± 0.1	−0.5
	10.0	0	77.2 (2) ± 0.2	83.4 ± 0.4	77.4 (2) ± 0.3	−2.2
	0	2.5	92.2 (1)			12.6
P. mirabilis						
6.5×10^{-5} M	0	0	66.0 (2) ± 0.9			
38% G·C[b]	2.5	0	78.1 (1)			12.1
	5.0	0	76.4 (1)			10.4
	10.0	0	73.3 (1)	80.0	73.5	7.5
d(A-T)	0	0	46.9 (3) ± 0.5			
6.5×10^{-5} M	2.5	0	58.3 (1)			11.4
	5.0	0	58.5 (1)			11.6
	7.5	0	59.3 (1)			12.4
	10.0	0	60.3 (1)			13.4
	0	2.5	59.5 (1)			12.6
	0	10.0	63.6 (1)			16.7

[a] The number in parentheses after the T_m value is the number of samples run. If values for more than one sample were determined, the average value is given. The average deviation is indicated by the plus or minus number. ΔT_m is the change in T_m observed in the presence of metal ion compared to the T_m in the presence of buffer alone. [b] Marmur and Doty (1962).

ported that the single-stranded synthetic polynucleotides poly(A) and poly(I) were precipitated in solutions of Mg^{2+} and Mn^{2+} of 1–5 mM independent of polynucleotide concentration (10–500 μg/ml). In order to avoid precipitation of the denatured DNA, metal ion concentrations of 1 mM and less were used. According to Eigner and Doty (1965) denatured DNA does not aggregate upon cooling for a number of hours in solutions of less than 50 μg/ml even in high salt concentrations (0.195 M Na^+). The DNA concentrations used here were 21–23 μg/ml.

Biphasic melting curves were observed when the DNAs, but not d(A-T), were heated in the more concentrated solutions of Mn^{2+}, but were not observed in the presence of similar concentrations of Mg^{2+} (Figure 1). In the samples in which a biphasic melting curve occurred, the first phase comprises 80–125% of the increase in absorption occurring in the absence of Mn^{2+}, while the second phase, occurring with a smaller slope, entailed an increase of an additional 22–39%.

Hypochromic Spectra. The hypochromic spectrum is a difference spectrum showing the increase in absorbance upon denaturation of the DNA and results from the increased absorbance of the chromophores of the base rings upon rupture of hydrogen bonds and/or stacking forces present in native DNA (Thomas, 1952). Hypochromic spectra of some of the samples used for melting temperature determinations are shown in Figure 2a–d. It may be noted that when Mn^{2+} was present during the denaturation, the hypochromic spectra of the DNAs were shifted, the new maxima occurring at longer wavelengths, while the total hyperchromicity increased over the entire spectrum. The shift in the hypochromic spectra and the increase in hyperchromicity were directly related to the Mn^{2+} concentration and to the (G + C) content of the DNA. In contrast, much smaller changes in the shape of the hypochromic spectra or in the total hyperchromicity were observed in the case of d(A-T) in the presence of Mn^{2+}, or with the DNAs or d(A-T) in the presence of Mg^{2+}.

No shift in spectra or increase in absorption occurred with native DNA at room temperature at $[Mn^{2+}]$:[DNA-P] ratios of 15 or lower. The decrease in thermal stability in the presence of Mn^{2+} may thus be due to a stabilization of denatured regions, rather than to a destabilization of the double helix. The increase in hyperchromicity, the shift in the hypochromic spectra, and the appearance of a biphasic curve when DNA is heated in the presence of Mn^{2+} could be explained by the interaction of Mn^{2+} with the base moieties as regions of the DNA become denatured.

TABLE II: Effect of Mn^{2+} on Line Widths of Protons of Several Dinucleoside Monophates.[a]

Proton	Metal: Ligand	Line Width	Metal: Ligand	Line Width	Δw	Metal: Ligand	Line Width	Δw
TpT (0.069 M)[b]								
H-6	0	3.0	0.0016	3.0	0	0.005	3.5	0.5
CH_3		3.0		2.5	0		3.0	0
ApA (0.01 M)								
H-8 5′	0	3.5	0.0024	4.0	0.5	0.004	6.0	2.5
3′		3.0		4.5	1.5		4.5	1.5
H-2 5′		2.0		3.5	1.5		3.0	1.0
3′		2.5		3.5	1.0		3.5	1.0
H-1′ 5′		2.5		3.5	1.0		3.0	0.5
3′		2.5		3.5	1.0		3.0	0.5
ApG (0.028 M)								
H-8 A	0	3.0	0.0006	5.0	2.0	0.0011	8.0	5.0
H-2 A		2.0		3.0	1.0		4.0	2.0
H-8 G		5.0		20.0	15.0		38.0	33.0
H-1′ A		2.0		3.0	1.0		4.0	2.0
H-1′ G		1.5		1.5	0		4.0	2.5
GpA (0.020 M)								
H-8 A	0	3.0	0.0007	4.0	1.0	0.0015	8.0	5.0
H-2 A		3.0		4.0	1.0		7.0	4.0
H-8 G		4.0		8.0	4.0		22.0	18.0
H-1′ A[c]		3.0		3.5	0.5		5.0	2.0
H-1′ G[c]		4.0		3.0	0		4.0	0
CpA (0.04 M)								
H-8A		2.5	0.0008	5.5	3.0	0.0012	7.0	4.5
H-2 A		2.0		4.0	2.0		3.5	1.5
H-6 C		1.5		1.5	0		2.0	0.5
H-1′ A		1.5		2.5	1.0		2.5	1.0
H-1′ C		1.5		4.0	2.5		5.0	3.5

[a] Solutions were adjusted to pH 7.0 ± 0.2. Spectra were run on the Varian HA-100 spectrometer. Metal:ligand ratios are in moles per mole, line widths are in cycles per second ±0.5, Δw is the increase in line width. [b] 5′-H and 3′-H peaks could not be distinguished in either nucleoside. [c] Doublets merged and were measured as one peak.

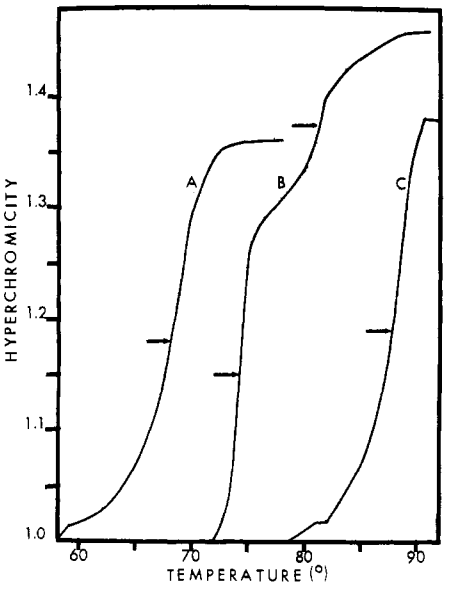

FIGURE 1: The effect of Mn^{2+} and Mg^{2+} on the heat denaturation of *E. coli* DNA. *E. coli* DNA was dissolved in 0.01 M Tris (pH 7.4) (A), 0.01 M Tris (pH 7.4) + 1×10^{-3} M Mn^{2+} (B), or 0.01 M Tris (pH 7.4) + 1×10^{-3} M Mg^{2+} (C). T_m values are denoted by arrows and were 68.1° (A), 74.2 and 80.8° (B), and 87.8° (C). Slopes were 4.1 (A), 0.6 and 2.3 (B), and 3.3 (C).

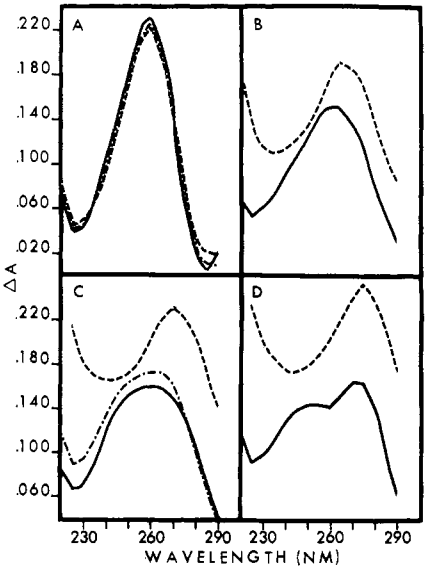

FIGURE 2: Hypochromic spectra of various DNAs and d(A-T) in 0.01 M Tris (pH 7.4) (——), 0.01 M Tris (pH 7.4) + 1×10^{-3} M Mg^{2+} (— – — –), or 0.01 M Tris (pH 7.4) + 1×10^{-3} M Mn^{2+} (– – –). d(A-T) (A), *P. mirabilis* DNA (B), *E. coli* DNA (C), and *M. lysodeikticus* DNA (D).

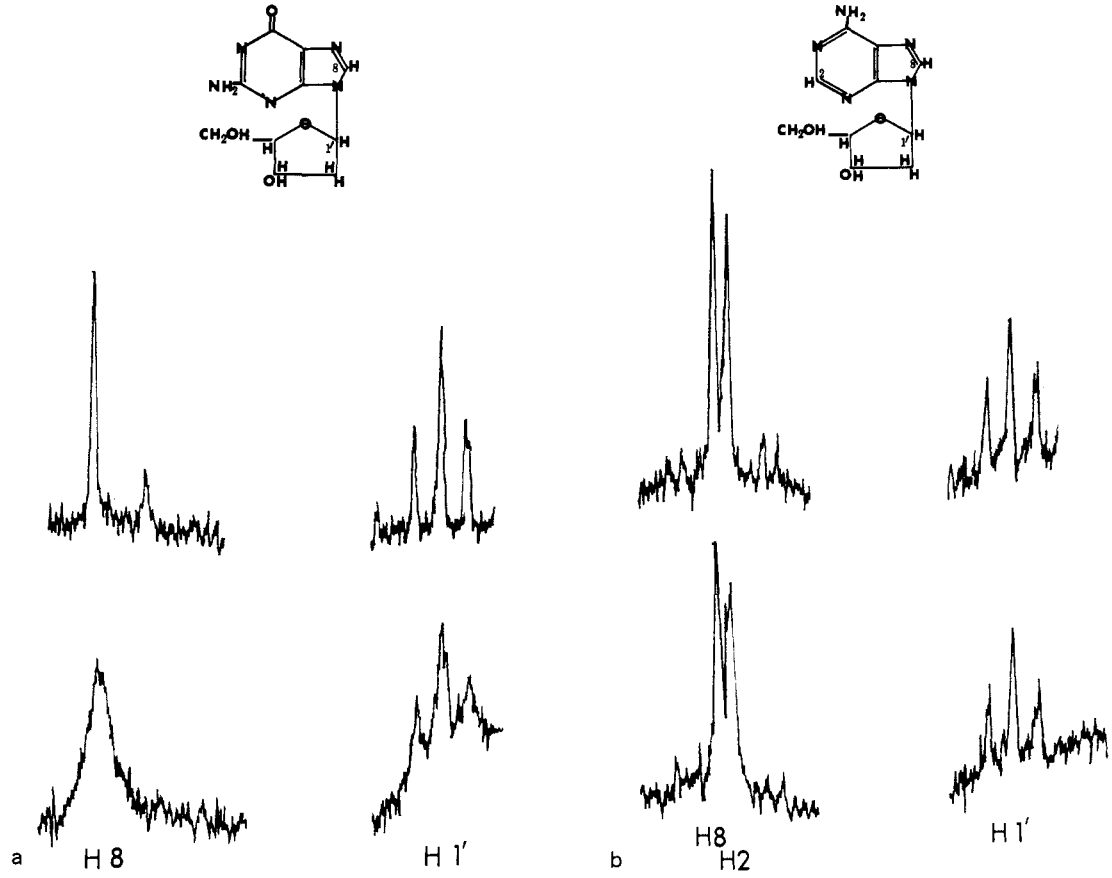

FIGURE 3: (a, left) The effect of Mn^{2+} on the resolved proton peaks of the nmr spectrum of deoxyguanosine. The upper tracings show proton peaks of deoxyguanosine without added metal ion and the lower tracings show these peaks in the presence of $MnCl_2$ at a metal:ligand molar ratio of 0.01. (b, right) The effect of Mn^{2+} on the resolved proton peaks of the nmr spectrum of deoxyadenosine. The upper tracings show the proton peaks of deoxyadenosine without added metal ion, and the lower tracings show these peaks in the presence of $MnCl_2$ at a metal ligand molar ratio of 0.01.

Nuclear Magnetic Resonance. Manganous ion is paramagnetic and as such exerts a large influence on the magnetic resonance signals of any nuclei in its proximity. Thus, nuclear magnetic resonance proves to be an excellent probe of complex formation by paramagnetic metal ions both as to magnitude and sites of binding. For a detailed treatment of this topic, particularly as it applies to systems similar to those reported here, the reader is referred to the paper by Glassman *et al.* (1971).

The results of the melting studies of DNA molecules in the presence of Mn^{2+} give some indication of a possible interaction between Mn^{2+} and guanine and/or cytosine. An ideal way to test for such an interaction would be to record the high-resolution proton nmr spectrum of DNA, identify the peaks due to the various bases, add Mn^{2+} and observe which peaks are selectively broadened. Unfortunately for a molecule as large and complex as DNA no such resolution is possible, hence we must study smaller fragments of the DNA molecule.

The dinucleoside monophosphates contain many of the features desired for such a study. The metal ion can undergo primary binding with the phosphate and two bases can be placed in direct competition with each other for any secondary association with the metal ion. Furthermore Ts'o *et al.* (1969) have assigned the proton peaks in the dinucleoside monophosphates so that it is easy to identify selective association of Mn^{2+} with any of the bases.

The effect of Mn^{2+} on the line widths of dinucleoside monophosphate proton signals is shown in Table II. In these cases the Mn^{2+}:dinucleoside monophosphate ratio is much less than one and the observed proton spectra are weighted averages of the spectra of complexed and uncomplexed ligands due to rapid ligand exchange between the two states. From the data of Table II it is clear than Mn^{2+} interacts magnetically much more strongly with the ring proton H-8 of deoxyguanosine than any other proton of any of the other nucleosides studied. Of itself, however, this does not indicate that Mn^{2+} is bound to the guanine ring in any way. As discussed by Glassman *et al.* (1971) the Mn^{2+} might be held in the vicinity of the guanine for conformational reasons having nothing to do with the Mn^{2+}–guanine binding. Selective broadening of the proton at C-8 of the adenosine 5′ nucleotide in ApA by Mn^{2+} is attributed by Chan and Nelson (1969) to the closer proximity of this proton to the phosphate group where Mn^{2+} is presumably bound. The much greater degree of broadening of the C-8 proton of the guanosine nucleotide, and the fact that this broadening occurs whether the guanosine is in the 3′ or 5′ position indicates that when guanosine is present Mn^{2+} is binding at a site closer to this proton than the phosphate group. A natural question which arises concerns the existence of a site on guanine for which Mn^{2+} would show some preference in comparison with sites on the other nucleoside bases in the absence of any conformational features having to do with the presence of the phosphate.

The answer to this question should be provided by a study of the broadening of the proton signals from the deoxynucleosides in the presence of Mn^{2+}. The effect of Mn^{2+} on the line

TABLE III: Effect of Mn^{2+} on Line Widths of Nucleoside Protons.[a]

Proton	Metal: Ligand	Line Width	Metal: Ligand	Line Width	Δw	Metal: Ligand	Line Width	Δw
dThd[b] (0.1 M)								
CH_3	0	1.0	0.005	1.5	0.5	0.01	2.5	1.5
H-6		1.0		3.0[d]			3.0[d]	
H-1′		1.5		1.5	0		2.0	0.5
dGuo[c,e] (0.05 M)								
H-8	0	1.0	0.0022	3.0	2.0	0.011	7.5	6.5
H-1′		1.5		2.0	0.5		3.0	1.5
Guo[c,e] (0.05 M)								
H-8	0	2.0	0.0024	3.0	1.0	0.006	6.5	4.5
H-1′		1.5		1.5	0		2.0	0.5
dAdo[c] (0.05 M)								
H-2	0	1.5	0.005	2.0	0.5	0.01	2.5	1.0
H-8		1.5		1.5	0		2.0	0.5
H-1′		1.5		1.5	0		1.5	0
Ado[c] (0.05 M)								
H-2	0	1.5	0.005	2.0	0.5	0.01	2.0	0.5
H-8		1.5		2.0	0.5		2.0	0.5
H-1′		1.0		1.5	0.5		2.0	0.5
dCyd[b] (0.01 M)								
G-5	0	1.5	0.011	2.0	0.5	0.026	3.0	1.5
H-6		1.5		2.0	0.5		2.5	1.0
H-1′		2.0		4.0	2.0		*f*	
Cyd[b] (0.1 M)								
H-5	0	1.5	0.011	3.0	1.5	0.026	3.0	1.5
H-6		1.5		3.0	1.5		3.0	1.5
H-1′		2.0		8.0[d]			*f*	

[a] Nucleoside solutions were adjusted to pH 6.8 ± 0.3, and were kept at 80°. Metal:ligand are in moles per mole. Line widths were measured in cycles per second ±0.5. Δw is the increase in line width. [b] Spectra run on Varian A-60A spectrometer. [c] Spectra run on Varian HA-100 spectrometer. [d] Peaks of doublet merged and measured as one peak. [e] Samples were dissolved in H_2O. [f] Peaks broadened and merged, could not be measured precisely.

widths of the resolved proton signals of deoxyguanosine and deoxyadenosine can be seen in the portions of the spectra that are shown in Figure 3a,b. Measurements of the line widths of the proton signals of various nucleosides examined in the presence and absence of Mn^{2+} are given in Table III. The *Chemical Abstracts* numbering system was used, and the structures are shown as inserts in Figure 3. As can be seen there was little or no broadening of the peaks of the protons on the base rings of thymidine, adenosine, or deoxyadenosine even at relatively high metal:ligand ratios, indicating that there was little interaction of Mn^{2+} with the base rings of these nucleosides at these concentrations. Both guanosine and deoxyguanosine showed strong preferential broadening of the proton at C-8 (H-8) as compared to that at C-1′ (H-1′), indicating that Mn^{2+} interacts at a specific site on the base ring. The nmr spectrum of a solution of deoxyguansoine and $MnCl_2$ was compared with that of the same solution diluted 1:1 with water. The line width of the H-8 signal decreased by a factor of two upon such dilution, indicating that a weak 1:1 complex was formed.

Discussion

The physical properties of DNA in solution were altered by the presence of millimolar concentrations of Mn^{2+} as compared to equivalent concentrations of Mg^{2+}. A decrease in the thermal stability of the DNA double helix with increasing concentrations of Mn^{2+} is clearly indicated by the melting temperature studies. The supporting electrolyte concentrations, 0.01 M Tris plus 0.2–1.0 mM $MnCl_2$, were in the range in which an increase of melting temperature has been observed using chloride salts of the alkali metal ions (Marmur and Doty, 1962; Owen *et al.*, 1969). Thus the destabilizing effect is not due to the anion, an effect which is seen at salt concentrations greater than 0.3 M (Hamaguchi and Geiduschek, 1962; Gruenwedel and Hsu, 1969).

The increase in T_m observed with increasing monovalent salt concentrations at low concentration is believed to be due to interaction of the cations with the DNA-P groups which neutralizes their negative charges and allows a more stable double helix to form (Schildkraut and Lifson, 1965). As Eichhorn and Shin (1968) originally reported, low concentrations of Mn^{2+} increase the thermal stability of DNA due to reaction with the phosphate groups, but as the concentration of Mn^{2+} is increased, the thermal stability decreases due to reaction with the bases. Our results are similar and in addition show that the decrease in thermal stability is dependent on the base composition of the DNA. Shifts in the hypochromic spectra, biphasic melting curves, and increased in hyperchromicity are also more evident as the (G + C) content of the DNA in-

creases and are not observed when d(A-T) is melted in the presence of Mn^{2+}. It appears that Mn^{2+} will interact with the base rings of guanosine and/or cytosine in the presence of phosphate groups which also presumably interact with the metal ion.

The nmr results for heteronucleotides containing guanosine show an obvious preferential broadening of the H-8 signal of the base ring of guanine, and the metal ion–base interaction is greater in the 5′-bound nucleoside than in the 3′-bound moiety. In ApG and GpA the metal ion–guanine interaction leads to an increase in the effect of the Mn^{2+} on the adenine over that observed in the ApA complex. If Mn^{2+} is binding at a site on the guanine ring in GpA and ApG, base stacking and the molecular conformations (T'so *et al.*, 1969) of the two dinucleosides might allow this bound Mn^{2+} to be sufficiently close to the adenosine to have a greater effect on the line widths of the ring protons than would a Mn^{2+} bound to the phosphate alone, as is presumably the case in ApA.

The results given in Tables II and III also show the dramatic effect phosphate binding has on ring binding. The Mn^{2+}–guanine interaction is much more pronounced in the heteronucleotide system than with the nucleoside. In the phosphate-containing species the primary metal ion association is presumably with the phosphate and the secondary ring association is then driven by the favorable entropy change which drives any chelation process. In the nucleoside complex there is no such favorable entropy for metal ion–base association and the interaction is consequently much weaker.

The experiments reported here indicate that Mn^{2+} complexes with electron-donor groups on DNA–guanine probably resulting in the stabilization of denatured regions of the DNA molecule. It does not appear that Mg^{2+} interacts with DNA in the same manner at the same concentrations. Thus, substitution of Mn^{2+} for Mg^{2+} in an enzymatic reaction involving DNA could result in (1) interaction of Mn^{2+} with DNA–guanine causing changes in local secondary structure or charge concentration, leading to new binding sites, and/or (2) interaction of Mn^{2+} with substrate or enzyme molecules increasing their affinity for (G·C)-containing sites in the DNA. In either case, the rate and specificity of a reaction could be altered. Such an effect has been observed in the binding of *M. luteus* DNA polymerase. Litman (1971) has shown that the enzyme binds more strongly to poly(dGdC) in the presence of Mn^{2+} than in Mg^{2+} but that enzyme binding to poly[d(A-T)] is enhanced to the same degree by the presence of either cation.

The binding of Mn^{2+} to nucleic acids has been studied by measuring the nuclear magnetic relaxation rate of the solvent water protons and by electron spin resonance signals of free Mn^{2+}. Studies of DNA have indicated that there are two types of Mn^{2+} binding sites on the DNA molecule; strong binding sites (equilibrium constant of about $10^4\ M^{-1}$, and about 0.2 site/phosphate residue) and weak binding sites (Eisinger *et al.*, 1965). Yeast RNA and tRNA also appear to have two types of binding sites, although the number of strong binding sites is lower than in DNA; 0.08–0.16 site/phosphate (Eisinger *et al.*, 1965; Bekker and Molin, 1969; Cohn *et al.*, 1969; Danchin and Guéron, 1970). The nature of the strong binding site in tRNA has been further investigated by Danchin and Guéron (1970). They suggest that each Mn^{2+} is bound by four ligands to tRNA, two of which may be phosphates and the remaining ones 2′-OH groups of the ribose moieties. Since DNA, which lacks the 2′-OH group, also has two classes of binding site, and the work presented here has indicated a preferential interaction of Mn^{2+} with guanine residues which are present in both nucleic acids, the possibility arises that some of the ligands at the strong binding sites may be a site on the base ring of guanine.

There are several obvious extensions of the work reported here. First there is a rather large gap between dinucleoside monophosphates and DNA. One wonders if the nmr studies would reveal a Mn^{2+} preference for guanine binding in ApApG, for instance, as compared to adenine binding in ApApA. Studies of this type can be made even in the absence of detailed peak assignments. Second we would like to characterize the Mn^{2+} binding site(s) much in the way that the location and type of ring binding were treated by Glassman *et al.* (1971). Research along these two lines is currently in progress.

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