Studies of Heavy Metal Binding with Polynucleotides Using Optical Detection of Magnetic Resonance. Silver(I) Binding

Sir:

The binding of metal ions by polynucleotides has been investigated by a variety of physical methods.[1] Silver(I) and mercury(II) are among the few ions which bind specifically to the heterocyclic bases of DNA, whereas most cations (such as Mg(II), Mn(II), and Co(II)) interact with the phosphates. The binding of Ag(I) with DNA is reversible, and two types of complexes have been studied.[2-4] Type I complexing of DNA occurs at extremely low Ag(I) concentrations, does not involve release of protons, and appears to be more important in GC-rich DNA's. Type II complexing is accompanied by proton release and appears to occur with both GC and AT base pairs. Type II complexes are suppressed at low pH and can be observed as a separate binding step in the potentiometric titration of DNA with Ag(I). At pH ≥7, type I and type II binding occur as a single step and are complete at $r_b \sim 0.5$, where r_b is the ratio of bound Ag(I) per nucleotide.

Recently, Rahn and Landry[5] have investigated the effect of Ag(I) binding with DNA and synthetic polynucleotides on their luminescence properties and on thymine photodimerization. They find up to a 20-fold enhancement of the rate of thymine dimerization in DNA and poly(dT) which parallels a similar enhancement of the phosphorescence quantum yield. The triplet lifetime at 77 K is reduced by over an order of magnitude, and the fluorescence is quenched. Such changes in luminescence properties are characteristic of an external heavy atom effect[6,7] and is good evidence that Ag(I) binds directly to the heterocyclic bases. Some time ago it was demonstrated[8,9] that paramagnetic resonance is useful for identifying the phosphorescent chromophores of DNA, since the zero-field splitting parameters D and E differ considerably between the heterocyclic base triplet states, while their phosphorescence spectra are quite similar. Thus the triplet state of DNA has been assigned to T with the aid of its characteristic D and E, using conventional EPR.[10] Although it would be desirable to identify the triplet state(s) of Ag(I)-complexed DNA using EPR, the enhanced triplet yield is more than compensated by the shortened triplet lifetime and results in undetectable triplet population in the steady state.[5] In this context, an advantage of optical detection magnetic resonance (ODMR) becomes apparent. The sensitivity of this method rests on the efficiency of conversion of microwave quanta to optical photons and is not tied down to the steady state triplet population as is conventional EPR. In Ag(I)-complexed DNA which might contain nonradiative as well as radiative triplet states during optical pumping, ODMR signals should be observed only from the radiative ones and should serve to identify the sites of Ag(I) complexing. An additional requirement for observing ODMR signals from the Ag(I)-complexed bases is the existence of a *selective* radiative heavy atom enhancement of the triplet sublevels; i.e., the radiative rate constants of one pair of sublevels (as well as their populations) must differ.[11] In this communication, we report ODMR measurements on several synthetic polydeoxyribonucleotides and calf thymus DNA complexed with Ag(I).

Measurements are made in zero field as described previously.[12] Phosphorescence decay measurements at 4.2 K show that essentially all the phosphorescence decays with a lifetime between 10 and 30 msec when $r \geq 0.1$. (r is the molar ratio of $AgNO_3$ added to the nucleotide, and should be near r_b for $r \leq 0.5$ under the experimental conditions.[2,3]) Slow passage signals are accumulated at 1.2 K using microwave sweep rates between 2 and 4 GHz/sec. Sublevel lifetimes are estimated for each slow passage signal by means of a microwave fast passage[13] through the same frequency region (sweep rate of ca. 100 GHz/sec). In each case a transient with a decay constant of ca. 10 msec is observed indicating that the signal originates from a heterocyclic base interacting with Ag(I).

ODMR signals observed from poly(dT) ($r = 1$) and from calf thymus DNA ($r = 0.5$) are shown in Figure 1. The frequencies at which ODMR signals are observed in the synthetic polydeoxyribonucleotides are given in Table I along with an assignment of $|D|/hc$ and $|E|/hc$. In each case the ODMR signal is an increase in phosphorescence in-

Table I. Optically Detected Magnetic Resonance Data of Silver Complexes of Synthetic Polydeoxyribonucleotides

Sample[a]	ODMR frequencies (GHz) and line widths (MHz) ν_1 ($\Delta\nu_1$)	ν_2 ($\Delta\nu_2$)	ν_3 ($\Delta\nu_3$)	Zero-field splitting parameters (cm^{-1}) ODMR $\lvert D\rvert/hc$	ODMR $\lvert E\rvert/hc$	EPR[b] $\lvert D\rvert/hc$	EPR[b] $\lvert E\rvert/hc$
Poly(dA)	1.63 (703)	2.66 (600)	4.36 (750)	0.117	0.028	0.121	0.027
						0.116[c]	0.027[c]
Poly(dT)	0.74 (143)	5.45 (297)	6.16 (310)	0.194	0.012	0.203	0.010
Poly(dG)	1.29 (221)	3.66 (497)	4.73 (385)	0.140	0.021	0.141	0.017
Poly(dC)	1.69 (349)	[d]	7.63(1300)	0.227	0.028	0.194[e]	
Poly(dA-dT)[f]	0.78 (187)	5.30 (152)	6.15 (304)	0.191	0.013		
Poly(dA, dT)[g]	1.63 (292)	2.12 (346)	3.91 (289)	0.102	0.028		
		5.42 (500)	6.24 (675)	0.195	0.014		
Poly(dG)·Poly(dC)[f]	1.18 (143)	3.54 (182)	4.75 (288)	0.138	0.020		

[a] $T = 1.2$ K. $r = 0.5$, except for poly(dA-dT) and poly(dT) where $r = 1$. [b] EPR data refers to uncomplexed mononucleotides unless noted. From J. Eisinger and R. Shulman, *Science*, 161, 1311 (1968). [c] Values for poly(A), ref 9. [d] Signal not observed. [e] Value for $D' = (D^2 + 3E^2)^{1/2}$ of CMP. [f] Duplex. [g] Random coil.

Table II. Optically Detected Magnetic Resonance of Silver Complexes of Calf Thymus DNA

r	ODMR frequencies (GHz) and line widths (MHz) ν_1 ($\Delta\nu_1$)	ν_2 ($\Delta\nu_2$)	ν_3 ($\Delta\nu_3$)	$\lvert D\rvert/hc$ (cm^{-1})	$\lvert E\rvert/hc$ (cm^{-1})	Assignment	R[a]
0.1	1.15 (171)	3.63 (200)	4.72 (362)	0.140	0.019	G	≥8
0.5	1.20 (220)	3.68 (300)	4.88 (507)	0.143	0.020	G	
		5.49 (730)	6.29 (633)	0.197	0.013	T	1.5
1.0	1.24 (372)	3.82 (319)	4.80 (404)	0.144	0.019	G	
		2.53 (686)				A	1.1
		5.68 (643)	6.21 (580)	0.198	0.009	T	

[a] Ratio of intensity of ν_3 signal of G to ν_3 signal of T.

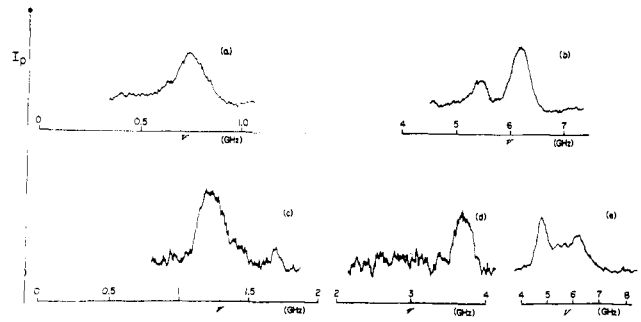

Figure 1. ODMR signals observed from Ag(I) complexes of (a) and (b) poly(dT); (c), (d), and (e) calf thymus DNA.

tensity showing that the steady state sublevel populations and radiative decay constants are inverted in order. It is apparent from Table I that binding of Ag(I) to the heterocyclic base causes little change in the zero-field splittings as determined by conventional EPR measurements of the triplet state of the uncomplexed molecule. Initial binding of Ag(I) to calf thymus DNA, $r = 0.1$, results in ODMR signals only from the triplet state of guanine indicating that the strongest binding (type I) results in Ag(I)-G complexes (Table II). Subsequent binding of additional Ag(I), $r = 0.5$, results in the appearance of ODMR signals due to the triplet state of thymine, showing that Ag(I)-T complexes form at higher r_b than the Ag(I)-G complexes. The thymine ODMR signals probably result from type II complexes. It has been suggested[3,4] that type II complexes result from replacement of a proton between T(N3) and A(N1) or C(N3) and G(N1) by Ag(I), leading to linear N-Ag-N bonds. This is consistent with our results, since A and T when both bound to Ag(I) should result in phosphorescence from only T, which has the lower energy triplet state. When Ag(I) is increased to $r = 1.0$, an additional ODMR signal is observed at 2.53 GHz with little change in the frequencies of the signals assigned to G and T. We have assigned this new signal to the triplet state of A.[14] We think that the A ODMR signal in DNA might result from partial strand separation caused by Ag binding—possibly to A(N7) which is already engaged in type II complexing. Triplet energy trapping on Ag-adenine complexes then would be possible.

If type I complexes have the "π-sandwich" structure previously suggested,[3,4] the absence of T signals at $r_b \sim 0.1$ can be explained by a stronger perturbation of Ag(I) on G than on T in the case of G-Ag-T complexes. This could result in a lower triplet energy for G and prevent the normally expected G → T triplet energy transfer.[15]

Acknowledgment. This work was supported by a grant from the National Institutes of Health (GM-21795).

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(14) Although only T signals are observed in the poly(dA-dT) duplex at $r \leq 1$, when $r = 10$ signals are observed from Ag–A complexes at 1.46, 2.58, and 4.00 GHz.
(15) The binding of Ag(I) to the bases is accompanied generally by a red shift of the optical absorption bands,[2] and we excite our samples to the red of the normal base absorption. We have found that Ag(I) binding to GMP results in a shift of the phosphorescence origin from 27.2×10^3 to 25.0×10^3 cm^{-1}, which is below the triplet energy of TMP (26.3×10^3 cm^{-1}).

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Received December 16, 1974

Hexakis(dimethylamido)ditungsten. The First Structurally Characterized Molecule with an Unbridged Triple Bond between Tungsten Atoms

Sir:

The great propensity of molybdenum to form M-M bonds of order 3 and 4 might naturally lead one to expect the same of tungsten.[1] This has not proved to be the case so far. Indeed, only $W_2(CH_2SiMe_3)_6$ has been prepared and reported to form crystals isomorphous to those of $Mo_2(CH_2SiMe_3)_6$.[2] The paucity of W-W multiple-bonded compounds raises interesting questions. If W-W multiple bonds are inherently weaker than those of molybdenum, what are the reasons? Or, have certain subtle factors in the coordination chemistry of tungsten thus far precluded their preparation?

We report here the preparation and characterization of $W_2(NMe_2)_6$. This work (i) provides the first X-ray structural characterization of a compound containing an unbridged W-W triple bond,[3] (ii) allows a direct comparison of the molybdenum and tungsten triple bonds in the compounds $M_2(NMe_2)_6$, and (iii) suggests answers to the above questions.

The preparation and characterization of $W(NMe_2)_6$ from the reaction of WCl_6 and $6LiNMe_2$ has previously been reported.[8] It was noted[8] that formation of $W(NMe_2)_6$ in the above reaction was always accompanied by some reduction of tungsten(VI) and, on the basis of analytical data, the reduced tungsten species was formulated as $W(NMe_2)_3$. Our recent characterization of $Mo_2(NMe_6)$[9,10] encouraged us to pursue synthetic routes to $[W(NMe_2)_3]_n$ since, clearly, this could answer important questions concerning W-W bonding. Since reactions involving WCl_6 and $6LiNMe_2$ led to some reduction of W(VI), reactions involving lower valent tungsten halides were expected to give only, or at least predominantly, $[W(NMe_2)_3]_2$. This was not the case. $WCl_4(THF)_2$[11] and $WCl_4(OEt_2)_2$[11] react with $4LiNMe_2$ (in THF-hexane) to yield $W(NMe_2)_6$ as the *only* isolable dimethylamide of tungsten; no $[W(NMe_2)_3]_n$ was obtained. The cluster compound WCl_2 reacts with $2LiNMe_2$ to give a mixture of $W(NMe_2)_6$ and $[W(NMe_2)_3]_n$, richer in $W(NMe_2)_6$ than many samples obtained from reactions involving WCl_6. However, we have found that if $WCl_4(OEt_2)_2$ is allowed to decompose in diethyl ether at room temperature for 1 hr under an atmosphere of nitrogen, and the resultant black sludge then treated with $3LiNMe_2$ (THF-hexane), a mixture of $[W(NMe_2)_3]_n$ and