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Spectroscopic Studies on the Effects of Aluminum Ion on Calf-Thymus DNA

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^{31}P-Nuclear magnetic resonance (NMR) and circular dichroism (CD) spectroscopy indicated that Al binding induces numerous reversible changes in DNA structure. Broadening of the phosphorus signal from the phosphate backbone along with a downfield shift and decrease in intensity indicated that phosphate may be the likely site of Al binding. ^{27}Al NMR studies indicate that while Al^{3+} binds to the phosphate oxygen, the hydroxylated species probably prefer other probable sites like DNA bases.

Little is known about the exact biological role of aluminium (Martin, 1986; WHO Technical Report Series, 1986). In recent years, aluminium is found to be associated with neuronal disorders like neurofibrillary degeneration and impairment of learning and memory (Martyn et al. 1989; Farrar et al. 1988). Aluminium ion (referred as Al hereafter) is found to form complexes with biomolecules like ATP, proteins and citrate (Martin, 1986). Some studies on the Al effect on DNA indicated Al binding to DNA in neurofibrillary tangle-bearing neurons (Crapper et al. 1980) and also alteration of the template activity of DNA in plants (Matsumoto et al. 1977; Marimura et al. 1978). Earlier Karlik et al. (1980) studied the interaction of aluminium species with DNA at Al/DNA ratios of 0-0.7 by thermal denaturation, circular dichroism and fluorescent dye binding. They reported a variation in the nature of interaction with DNA by Al depending on the pH and concentration. Since no report is available on the effect of higher concentrations of Al on DNA structure, the present studies on Al-DNA interaction at higher concentration ratios (Al/DNA>40) were initiated and the results are reported in this paper.

MATERIALS AND METHODS

Calf-thymus DNA from BDH (London), aluminium acetate (Basic) and aluminium nitrate from BDH and ethidium bromide from Sigma (USA) were used.

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The concentrations of calf-thymus DNA used for ultra-violet and circular dichroism were 2.42×10^{-4} M in 0.1 M acetate buffer pH 4.6. Unless otherwise stated, acetate and phthalate buffers refer to sodium acetate and potassium phthalate throughout the text. The concentrations of DNA solutions were determined by measuring the absorbance at 260 nm (molar extinction coefficient = 7100) and are mentioned throughout in terms of a nucleotide residue. Stock solutions of 0.01 M aluminium acetate (basic) in the above mentioned buffer were prepared, from which additions were made to the DNA solutions. Aluminium acetate solution was clear without any precipitation.

At room temperature, the ultraviolet spectra and circular dichroism spectra were recorded on a Varian UV spectrophotometer and a JASCO J20 spectrophotometer respectively. The spectra at each concentration were the average of three recordings. Mean molar ellipticity [θ] values were calculated as described previously (Divakar et al. 1987).

^{31}P and ^{27}Al NMR spectra were recorded on a Varian Associates FT-80A NMR spectrometer operating at 32.203 MHz for ^{31}P and 20.72 MHz for ^{27}Al. The samples were locked externally, and proton decoupled spectra were obtained. A spectral width of 4000 Hz was used, and about 2000 scans were accumulated to get a reasonable spectrum for both ^{31}P and ^{27}Al NMR. About 10 mg/ml of sonicated DNA in the above mentioned buffer was used for ^{31}P NMR. A 0.03 M Al solution was used for ^{27}Al NMR into which sonicated DNA from a stock solution of 100 mg/ml was added in increasing amounts, and spectra recorded. A 60-70^{o} pulse with a total recycle time of 1 sec was employed in both cases. Samples were referenced from external 85% phosphoric acid for ^{31}P and $Al(NO_3)_3.9H_2O$ for ^{27}Al NMR to within $\pm$0.05 ppm.

Sonicated DNA was prepared according to Divakar et al. (1987). Binding constant values were determined by the method of Formoso (1972). Fluorescence emission was recorded with a Perkin Elmer 203 fluorescence spectrometer. Excitation of a equimolar DNA-ethidium bromide solution was done at 525 nm, and emission was monitored at 600 nm.

RESULTS AND DISCUSSION

^{31}P-NMR spectrum of calf-thymus DNA could not be recorded satisfactorily because of very high molecular weight. Hence, the calf-thymus DNA was sonicated to get fragments of a size of approximately 150$\pm$50 base pairs. Sonicated DNA gave a single signal for the phosphorus of the phosphate diester linkage with a $\nu_{\frac{1}{2}}$ (line-width at half-height) value of 50 Hz and a chemical shift value of 2.21 ppm (from H_3PO_4 - Fig-1 and Table-1). On addition of aluminium ion, significant changes were observed in the chemical shift as well as $\nu_{\frac{1}{2}}$ values. In the concentration range 0.001 to 0.01 M of Al, the signal not only showed a down-field shift (of about 0.52 ppm) with increasing Al concentration but also broadened significantly ($\nu_{\frac{1}{2}}$ = 80 Hz). Also the intensity

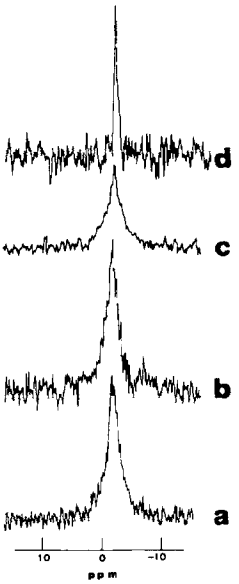

Figure 1. ^{31}P-NMR spectra of sonicated DNA obtained at 32.203 MHz showing the effect of Al addition to DNA-0.061 M in 0.1 M acetate buffer pH 4.6
a. 0 M; b. 0.001 M Al; c. 0.005 M Al; d. 5 mg EDTA added to 0.01 M Al

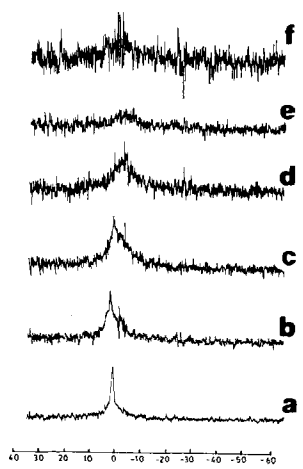

Figure 2. ^{27}Al NMR studies of addition of sonicated DNA to $Al(NO_3)_3$. DNA additions were carried out from a stock solution of 0.3 M concentration of Al in 0.1 M phthalate buffer pH 4.6. DNA additions were made to 0.05 M $Al(NO_3)_3$ in the same buffer. Concentrations of DNA are a. 0 M; b. 0.007 M, c. 0.014 M; d. 0.027 M; e. 0.039 M; f. 0.5 M.

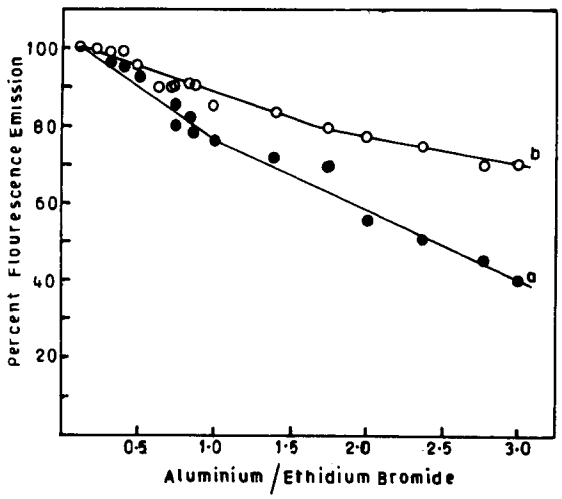

Figure 3. Decrease in percentage of fluorescence emission at 600 nm of an equimolar (2.42×10^{-4} M) solutions of DNA-ethidium bromide on adding Al. Al additions were carried from a stock solution of 2.67×10^{-3} M. (a) pH 4.0; (b) pH 7.0

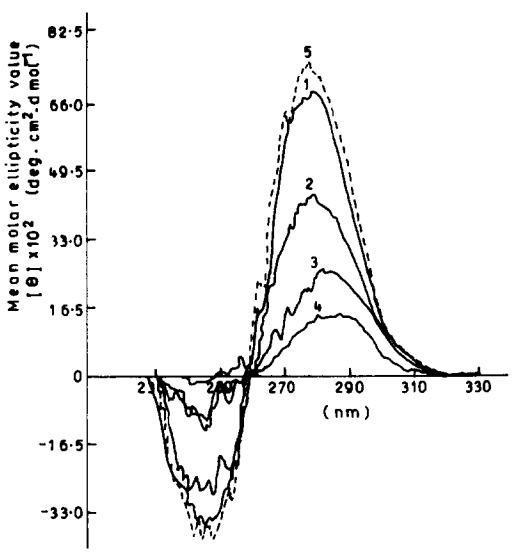

Figure 4. Effect of aluminium on circular dichroism spectra of calf-thymus DNA. DNA (2.42×10^{-4} M) in 0.1 M acetate buffer pH 4.6.
1. 0 M; 2. 0.0001 M; 3. 0.005 M; 4. 0.01 M; 5. 5 mg EDTA added to 0.01 M Al.

of the signal decreased indicating that Al binds in the vicinity of the phosphorus atom. The most likely position will be the phosphate oxygen of the backbone phosphate groups. The pK value of the phosphodiester group in DNA is$\simeq$1.5 (Ts'O, 1974), and hence at pH 2.0 this group will be in the monoanionic form capable of binding metal ions like Al. The observed broadening and decrease in intensity of the ^{31}P signal indicate that probably more than one Al species binds to DNA.

In ^{27}Al NMR, $Al(NO_3)_3.9H_2O$ gave a sharp signal at pH 4.0. At higher Al/DNA ratios at this pH value, a broadened envelope showed emergence of two signals: one at the same initial position as $Al(NO_3)_3$, and another slightly upfield (Fig-2, Table-1). With DNA addition, most of the peaks shifted upfield with increased broadening. In the DNA concentration range 0.0073 M to 0.5 M, the Al signals spread from 0-4.8 ppm. The $\nu_{\frac{1}{2}}$ values also ranged from 32.6 Hz for free Al^{3+} to 237.2 Hz for sample with a DNA concentration of 0.027 M. Also, it was observed that the peak at 0 ppm broadened immediately after DNA addition and another peak at 3.4 ppm appeared to broaden at higher DNA concentration with possible further splitting. This could not be studied satisfactorily because of an increase in the noise level. Progressive precipitation occurred on addition of DNA to Al solutions. The observed broadening at higher Al/DNA ratios indicated relative intermediate rates of exchange of Al species to DNA, and the emergence of a signal at 3.4 ppm indicated specific binding of Al to DNA at a slower rate of exchange on the NMR time scale. This differential binding might be due to specific species of Al binding to defined sites on DNA. The signal at 3.4 ppm increased in intensity with further splitting as the Al/DNA ratio decreased indicating that either binding to one particular site was preferred as the DNA concentration increased or selective binding of specific species of Al to DNA took place. $Al(NO_3)_3$ at pH 4.0 showed the following proportion of the hydroxylates species: Al^{3+} : $Al(OH)^{2+}$: $Al(OH)_2^{+}$: 85.5: 9.4: 5.1 (Bertsch et al. 1986). Since Al^{3+} is in major proportion at pH 4.0, its binding to phosphate oxygen may be responsible for the signal at 3.4 ppm.

Adding increasing concentrations of Al to an equimolar solution of DNA-ethidium bromide resulted in displacement of ethidium bromide by Al as indicated by the drop in fluorescence emission of DNA-ethidium bromide solution (Fig-3). This was observed at pH 4.0 and 7.0, although the magnitude of changes at the latter pH value was less than that at the former.

Decrease in the magnitude of positive and negative CD bands at 276 nm and 246 nm, respectively, were observed with increase in concentration of added Al (Fig-4). Mean molar ellipticity values determined for various concentrations of Al are given in Fig-4. The B form of DNA usually exhibits equal magnitude of positive and negative bands (Ivanov et al. 1973). Unwinding of the DNA helix probably gave rise to the observed fall in mean molar ellipticity values on adding Al. Several monovalent and divalent metal ions have also been shown to cause similar effects (Ivanov et al. 1973). Ultraviolet spectroscopic measurements also showed

Table-1. ^{31}P and ^{27}Al NMR studies on effects of addition of calf-thymus DNA

Sl. No.	^{31}P NMR*			^{27}Al NMR*		
	Al concentration in M	Chemical shift (δ)	Peak width at half height ($\nu_{1/2}$) in Hz	DNA concentration in M	Chemical shift (δ)	Peak width at half height ($\nu_{1/2}$) in Hz
1.	0	2.21	50	0	0	32.6
2.	0.001	2.40	60	0.0073	0, 3.4**	65.1**
3.	0.005	2.05	76	0.0142	0, 4.1	167.4
4.	0.010	1.69	80	0.0272	0, 3.7, 4.8	237.2***
5.	0.010+5 mg EDTA	2.19	18	0.039	--	--
				0.5	--	--

* Aluminium concentration used was 0.27 M in 0.1 M phthalate buffer pH 4.0. Sonicated DNA concentrations for both ^{31}P and ^{27}Al NMR were calculated for a mean residue molecular weight of 330 with DNA concentration for ^{31}P NMR at 0.061 M in 0.1 M acetate buffer pH 4.6. The $\nu_{1/2}$ value for the reference phosphoric acid is 20 Hz

** The chemical shift value at 0 refers to $Al(NO_3)_3$ and the other values to the shifts observed on DNA addition. Also the $\nu_{1/2}$ values given are for the major peak at 0

*** values for both peaks

-- could not be measured accurately due to broadening

a drop in absorbance by 0.35 units as the Al concentration was increased. The CD titration plot indicated that as many as five Al ions bind to DNA under the experimental conditions employed. Also, the determined binding constant value $2.3 \times 10^3 \pm 500$ M^{-1} represents the overall binding for the various species of Al present in the solution. At concentrations of Al greater than 0.01 M, precipitations in DNA solutions were observed. Addition of EDTA to the DNA-Al solution restored the original CD spectral characteristics of the calf-thymus DNA indicating that Al binding is reversible (Fig-4). EDTA binds to Al^{3+} much more tightly (binding constant value = 1.29×10^{16} M^{-1}) (Lange's Handbook of Chemistry, 1973) than DNA and hence, removes Al bound to DNA, effectively restoring the original spectral features of DNA. This may also indicate that DNA is still double stranded and intact after Al binding.

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