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Rapid Communication

Synthesis, characterization and nuclease activity of copper(II), nickel(II) and iron(II) complexes of 2-acetylpyridine oxime

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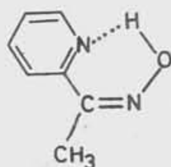
A few complexes of Cu(II), Ni(II) and Fe(II) with 2-acetylpyridine oxime have been synthesized and characterized by elemental analysis, IR, UV-vis spectroscopy, magnetic moments and cyclic voltammetry and their interactions with plasmid DNA are investigated.

Studies of chemical modification of nucleic acids by transition metal complexes are of paramount importance for the designing of chemotherapeutic drugs, regulating gene expression and designing tools for molecular biology[1-5]. Many coordination compounds of transition metal ions accomplish nucleolytic cleavage, the most important examples being Cu(II)-1,10-phenanthroline[6], Fe(II)-bleomycin[7], Fe(II)-EDTA[8], metalloporphyrins[9], Ru, Rh and Re complexes of polypyridyl ligands[10], Ni complexes of azamacrocycles[11] and Cu(II), Co(III) and Ni(II) complexes of desferrioxamine B[12]. Recently, Griffin *et al.*[13] have reported that $[(N,N'$-ethylene bis salicylideneaminato) $Mn(III)]^+$ mediates the cleavage of right handed double helical DNA in the presence of oxidants. Similarly, the photolytic cleavage of DNA by $[Au_3\{bis(dimethylphosphinomethyl)methylphosphino\}_2]^{3+}$ and methyl radical initiated DNA cleavage by Co(III) cyclam complex has been reported by Yam *et al.*[14] and Riordan and WEi[15] respectively. In another report, double stranded cleavage of pBR322 by the diiron complex $[Fe_2$ (N, N, N'N'-tetrakis (2-benzimidazolyl methyl)-2-hydroxy-1, 3-diaminopropane) $(OH)(NO_3)_4]$ have been described via hydrolytic mechanism induced by H_2O_2 or O_2 (ref.16). This continued interest in the transition metal complexes as chemical nucleases and the recent report of nucleolytic activity of hydroxamic acid in the presence of metal ions[17] prompted us to investigate the structural peculiarities of Ni(II), Cu(II) and Fe(II) complexes of 2-acetylpyridine oxime (**I**) (ACPYOX) and their DNA cleaving activities against plasmid pBR322.



ACPYOX (I)

Experimental

2-Acetylpyridine oxime (**I**) was prepared by adding a solution of hydroxylamine hydrochloride (5 mmol) and sodium hydroxide (5 mmol) in 50 ml aqueous methanol to a boiling solution of 2-acetylpyridine (5 mmol). The mixture was refluxed for 2 h. On cooling, a white crystalline product was obtained which was filtered and recrystallized from methanol (m.p. = 121°C). The metal complexes were prepared by adding the methanolic solutions of 2-acetylpyridine oxime (1.5 mmol) and sodium methoxide (1.5 mmol) to the aqueous solutions of the corresponding metal sulphates (0.5 mmol) with constant stirring for half an hour. The reaction mixture was set aside overnight in the refrigerator to yield a product which was washed with water followed by *n*-hexane and dried *in vacuo*.

Results and discussion

On the basis of elemental analyses, all chelates are assigned a metal to ligand stoichiometry of 1:3 except for the copper compound which has 1:2 metal to ligand stoichiometry (Table 1). The significant infrared absorption peaks and their assignments are also shown in Table 1. The free ligand exhibits a broad band in the range 3200-3400 cm^{-1} with multiple maxima which can be assigned to the intramolecularly H-bonded oximino hydroxyl group. On complexation, these bands are not much affected indicating that the hydroxyl group of the C=N−OH chromophore does not take part in coordination. The C=N acyclic and N−O stretching vibrations seen in the free ligand at 1580 and 950 cm^{-1} are shifted to higher frequencies in the complexes and appear at 1600-1620 and 1080-1090 cm^{-1} respectively which is an indication of neutral oxime group coordination[18]. The coordination of the pyridine nitrogen atom is indicated by the shift in the heterocyclic ring vibrations from 630-650 cm^{-1} upon complexation as observed for other metal-pyridine complexes[19] and is ascribed to the steric interactions of the heterocyclic rings within the coordination

Table 1—Characterization and electrochemical data of the complexes of 2-acetylpyridine oxime

Compound	Found (Calcd), %			μBM_{RT}	Significant I.R. bands (cm^{-1})			$E_{1/2}$ $M^{2+/3+}$	% electrochemical reversibility
	C	H	M		ν(O-H)	ν(C=N)	ν(N-O)		
ACPYOX	61.69 (61.75)	5.86 (5.92)	—	dia.	3200-3400	1580	950	—	—
$Fe(ACPYOX)_3SO_4$	48.96 (49.99)	4.53 (4.80)	10.89 (11.06)	4.95	3220	1605	1075	+0.71	98
$Ni(ACPYOX)_3SO_4$	44.35 (44.78)	4.18 (4.30)	10.26 (10.42)	3.05	3200	1600	1085	+0.32	100
$Cu(ACPYOX)_2SO_4$	38.06 (38.93)	3.58 (3.73)	14.19 (14.71)	1.70	3330	1605	1040	+0.26	89

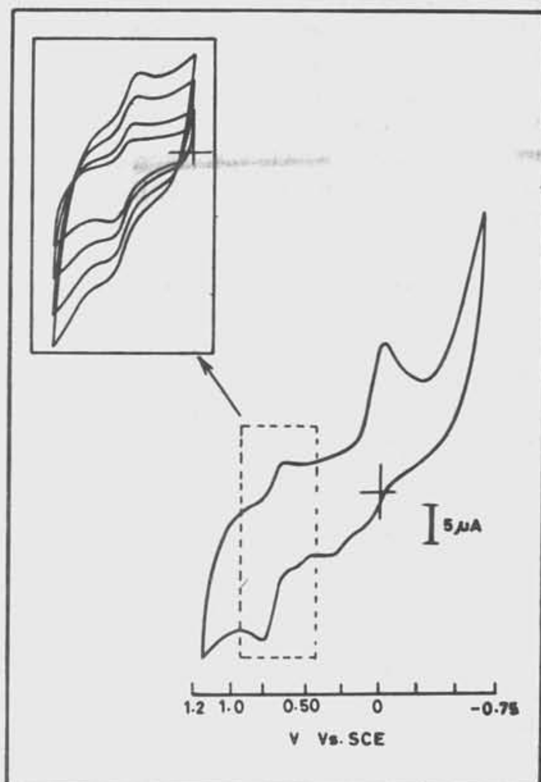


Fig. 1—Cyclic voltammogram of a 10 m*M* solution of $Fe(ACPYOX)_3SO_4$ complex in DMF with TEAP as supporting electrolyte. Inset shows the scan rate dependence.

sphere. The electronic spectra of the complexes are typical of an octahedral coordination in iron and nickel compounds while a square planar environment is suggested for the copper complex. The room temperature magnetic moments conform well with such formulations.

A representative cyclic voltammogram of the Fe(II) complex of 2-acetylpyridine oxime with the scan rate dependence as inset was recorded on a bioanalytical system CV-27 using a three-electrode configuration: Platinum; working saturated calomel electrode; reference and platinum; auxilliary with tetraethylammonium perchlorate as supporting electrolyte (Fig.1). The cyclic voltammetric profile of the ligand exhibits an irreversible reduction peak at −1.05V vs SCE which is assigned to the reduction of −C=N functionality[20]. Upon metal complexation, another one electron redox peak is seen in the resulting complexes which is reversible (Table 1) corresponding to the $M^{2+} \rightleftharpoons M^{3+}$ couple. A comparison of the $E_{1/2}$ values of this redox couple of the present complexes with other analogous nitrogen donor macrocycles[21] reveal that the oxime complexes undergo more facile redox changes which seems to be a requirement for the DNA cleavage by such nitrogen ligands according to Burrows *et al*[22].

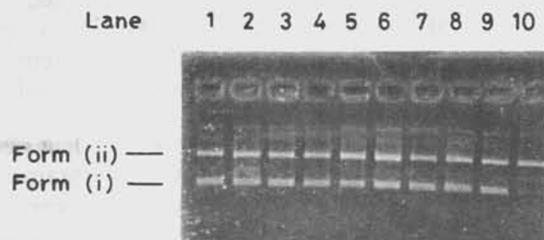


Fig. 2—1% Agarose gel showing the results of electrophoresis of pBR322 plasmid DNA (~4.3 kb); DNA (300 ng); metal complex (100 μ*M*) in DMF H_2O_2 (18 μ*M*); Incubation (37°C/30 min). Sterile deionised water was added to make final volume 20 μL.
lane 1 DNA; lane 2 DNA + H_2O_2; lane 3 DNA + ACPYOX; lane 4 DNA + ACPYOX + H_2O_2; lane 5 DNA + $Ni(ACPYOX)_3SO_4$; lane 6 DNA + $Ni(ACPYOX)_3SO_4$ + H_2O_2; lane 7 DNA + $Cu(ACPYOX)_2SO_4$; lane 8 DNA + $Cu(ACPYOX)_2SO_4$ + H_2O_2; lane 9 DNA + $Fe(ACPYOX)_3SO_4$; lane 10 DNA + $Fe(ACPYOX)_3SO_4$ + H_2O_2.

A gel electrophoresis experiment using pBR322 a circular plasmid DNA was performed with the present complexes in the absence and presence of H_2O_2 as an oxidant. From the results of the above experiment shown in Fig.2, it can be observed that at micromolar concentrations in the presence of oxidant, only $Fe(2ACPYOX)_3SO_4$ complex af-

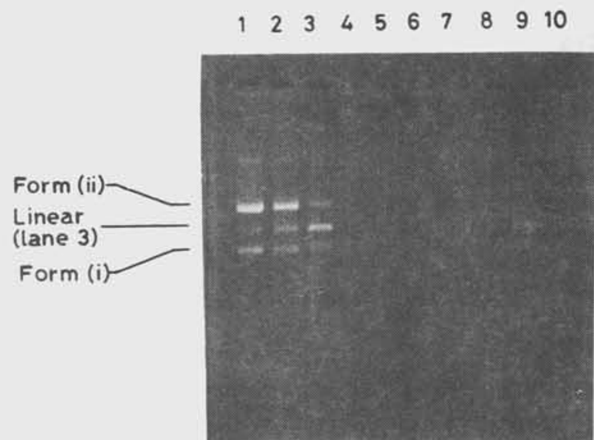


Fig. 3—1% Agarose gel electrophoresis showing the results of incubation of linear pBR322 plasmid with [Fe(ACPY-OX)$_3$SO$_4$] in the presence of H_2O_2 for different time periods. lane 1 DNA; lane 2 DNA; lane 3 linearized pBR 322 DNA; lane 4, 5, 6, 7, 8, 9, 10 DNA + Fe(ACPYOX)$_3$SO$_4$ + H_2O_2 at 60, 50, 30, 15, 10, 5 minutes respectively.

fords a discernible DNA cleavage at 30 min incubation period, as evidenced by the disappearance of form I (supercoiled) of plasmid. This is due to stepwise conversion of form I (supercoiled) plasmid DNA to form III (linear) through the transient formation of form II (open circular). This is consistent with the increased production of radicals mediated by ferrous ions by the well known Fenton reaction[23]. Incubating further (>40 min) results in a complete degradation of DNA. This is probably due to a single cut in circular DNA giving rise to a linear form having open ends and eventual fragmentation of plasmid DNA. To ascertain the role of open ends in facilitating DNA cleavage by iron complex, another experiment was carried out. Previously linearized plasmid DNA (pBR322) was subjected to the same experimental conditions as shown in Fig. 2. An appropriate amount of DNA was digested with restriction enzyme Hind III resulting in a single linear fragment (Fig. 3; lane 1). This linear DNA was purified (phenol-extraction followed by ethanol precipitation), dissolved in distilled water and diluted to a final concentration of 100 ng/μl. The purified linearized DNA (300 ng) was incubated with Fe(2ACPYOX)$_3$SO$_4$ (100 μM) in the presence of H_2O_2 (18 μM) for different time intervals. It can be seen (Fig. 3) that incubation for 5 min (Lane 9) results in a diffuse band indicating partial fragmentation of linear DNA while incubation for 10 min leads to a complete degradation (Lane 8). These results clearly reveal that the formation of open ends facilitates cleavage and resembles the exonuclease activity having a similar requirement.

The present studies have thus been able to establish that simple inorganic complexes with appropriate overall geometries, charge, reversible redox couples can be tailored to produce synthetic chemical nucleases having specific nucleolytic activity.

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