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Effect Of Photocleavage And Dna Binding Of Metal Complex Ru (Ii)1,10-Phenanthroline Complex And Their Actions

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Abstract

It is found that metal complexes are useful in designing of new drugs and also help in their synthesis. The fact is based on the concept of binding of metal complexes as well as photo cleavage. The paper focuses on the study of metal complexes that, how are they useful in the synthesis of new drugs, restriction enzyme and D.N.A. foot printing.

Keywords- Photo cleavage, D.N.A. binding, Shimadzu spectrophotometer and metal complexes.

Introduction

Metal complex have been found to be useful in designing and development of new drugs, restriction enzymes, D.N A. foot printing etc. and also their ability to probe the structure of D.N.A. itself. This is because of their potential to bind D.N.A. via a multitude of interactions and to cleave the duplex by virtue of their intrinsic chemical, electrochemical and photochemical reactivity. Ru(1,10-phenanthroline) is an example of this kind of metal complex. Variation of ligand as well as metal ion also effect the ability to bind with D.N.A. and photo cleavage of D.N.A. modification in metal complex of type $[M(phen)(LL)]^{n+}$ are well suited for this purpose, where M is metal ion, LL is a modified phenanthroline ligands. Comparison of D.N.A.binding and photo cleavage is characteristic of a family of [M(phen)LL] type complex.

Various physicochemical method and biochemical technique including UV/visible and viscometric titration, thermal denaturation, differential pulse voltametry and gel electrophoresis have been utilized to probe the nature of these complex.

Experimental

The required chemical of [M(phen)LL] were purchased from BDH (Mumbai, India). Deionized, triply distilled water was used for preparing various buffers. Calf thymus DNA, DABCO, SOD, PBN, D_2O, TBACl, pBR322DNA, EtBR are obtained from various laboratories of India. The supercoiled pBR 322 DNA (CsCl purified, Bangalore Genie, Bangalore, India) was used as received. Agarose (molecular biology grade) and ethidium bromide (EtBr) were purchased from Merck India.

Synthesis of ligands

All the required metal complex was synthesized according to literature and these complexes are analysed by elemental analysis to give correct molecular formulae. Before use they were dried in vacuum.

Method of Analysis

Shimadzu spectroscopy: Shimadzu model 530FTIR spectrophotometer was used to record UV and IR spectra.

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(1) Physical methods:

Shimadzu model UV-160 A (coupled with a temperature controller Model TCC-240A) and a JASCO Model 5300 FT-IR spectrophotometer, was used to record UV/Visible and IR spectra respectively. Fluorescence spectra were recorded with a JASCO Model 7700 spectrofluorometer for solutions having an absorbance less than 0.2 at the excitation wavelength. $[Ru(phen)_3]^{2+}$ was used as the standard for this purpose (/ = 0.028 in CH_3CN) . ^1H NMR spectra were recorded with a Bruker NR-FT 200 spectrometer using DMSO-d_6/$CDCl_3$ as the solvent and tetramethylsilane (TMS) as an internal standard.

ESR spectra were recorded with JEOL JM-FE3X spectrometer with diphenylpicrylhydrazide (DPPH) as an ESR standard. Magnetic susceptibility measurements for solid samples of the complexes were carried out using a Cahn Instrument (Model 6612) system. $CuSO_4$ and $Hg[Fe(CNS)_4]$ were employed as magnetic susceptibility standards. Diamagnetic corrections to the apparent magnetic susceptibility values have been incorporated, as specified. Cyclic voltammetric and differential pulse voltammetric experiments were performed on a Princeton Applied Research (PAR) 174A polarographic analyzer coupled with a PAR 175 universal programmer and a PAR RE 0074 *X*-*Y* recorder.

A platinum-button working electrode, a platinum-wire counter electrode and a saturated calomel reference electrode (SCE) were employed for experiments involving the non-aqueous solvents (PF_6 salts of the complexes). The SCE was separated from the deaerated (N_2) bulk electrolytic solution by a fritted-glass-discjunction containing the solvent (CH_3CN) and the supporting electrolyte ($TBAPF_6$). Ferrocene was used as an internal standard for these experiments.

(2) Absorption titration experiments

These experiments were performed by maintaining a constant concentration of the complex while varying the nucleic acid concentration. This was achieved by dissolving an appropriate amount of the metal complex in the DNA stock solution and by mixing various proportions of the metal complex and DNA stock solutions while maintaining the total volume constant (1 ml). This resulted in a series of solutions with varying concentrations of DNA but with a constant concentration of the complex. The absorbance (*A*) of the most red-shifted band of each investigated complex was recorded after successive additions of CT DNA. The intrinsic binding constant K_b, was determined from the plot of [DNA]/($\sum_a$–$\sum_f$) vs [DNA], where [DNA] is the concentration of DNA in base pairs, $\sum_a$, the apparent extinction coefficient is obtained by calculating A_{obsd}/[complex] and $\sum_f$ corresponds to the extinction coefficient of the complex in its free form. The data were fitted to (1) where $\sum_b$ refers to the extinction coefficient of the complex in the fully bound form.

$$[DNA]/(\sum_a-\sum_f) = [DNA]/ (\sum_b-\sum_f) + 1/K_b(\sum_b-\sum_f). \quad (1)$$

Each set of data, when fitted to the above equation, gave a straight line with a slope of $1/(\varepsilon_b–\varepsilon_f)$ and a *y*-intercept of $1/Kb(\varepsilon_b–\varepsilon_f)$. K_b was determined from the ratio of the slope to intercept.

(3)Studies related to DNA

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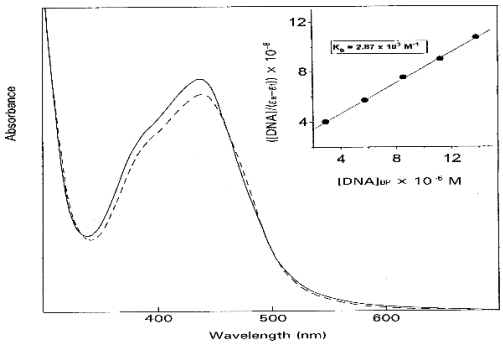
CT DNA concentration was measured by using its known extinction coefficient at 260 nm (6600 M^{-1} cm^{-1}) . BufferA (5 mM *tris*, *p*H 7·1, 50 mM NaCl) was used for absorption titration experiments and luminescence measurements, buffer B (1 mM phosphate, *p*H 7·0, 2 mM NaCl) was used for thermal denaturation and differential-pulse voltammetric experiments. BufferC (1·5 mM Na_2HPO_4, 0·5 mM NaH_2PO_4, 0·25 mM Na_2EDTA, *p*H = 7·0) was used for the viscometric titrations. The chloride salts of the complexes were used in studies with DNA.

(4)Thermal denaturation studies

The experiments related to DNA melting were carried out by monitoring the absorption (260 nm) of CT DNA (160 μM) at various temperatures, in both the absence and the presence (0–10 μM) of each investigated complex. The melting temperature (T_m) and the curve width μ_T (temperature ranges 10% to 90% of the absorption increase occurred) were calculated. The shape of the melting curves, T_m and μ_T values for CT-DNA and for CT-DNA in the presence of $[Ru(phen)_3]^{2+}$ were consistent with the literature data . Some of the metal complexes were seen to absorb at 260 nm, but control experiments suggested that this absorption is independent of temperature.

(5)Viscometry

Viscometric titrations were performed with a Cannon-Ubbelohde viscometer at 25 ± 1°C. Each compound (3–40 μM) was introduced into the degassed DNA solution (300 μM in base-pairs) present in the viscometer using a Hamilton syringe fitted with a glass extender. Mixing of the drug and DNA was done by bubbling with nitrogen. Flow times were measured, using a digital stop-watch, at least thrice and were accepted if they agreed within 0·1s. Reduced specific viscosity was calculated according to Cohen and Eisenberg. Plots of η/η_o (η and η_o are the reduced specific viscosities of DNA in the presence and absence of the drug) versus [drug]/[DNA] were constructed. Plot of η/η_o versus [EtBr]/[DNA] was found to be similar to that reported in the literature .



UV/Visible spectra of $[Ru(phen)2(phen\text{-}dione)]^{2+}$ (10 mM) with (- - - -)and without (_____) CT-DNA (100 mM in base-pairs) in buffer A. Inset: Plot of [DNA]/(e*a*–e*f*) vs [DNA] for this interaction

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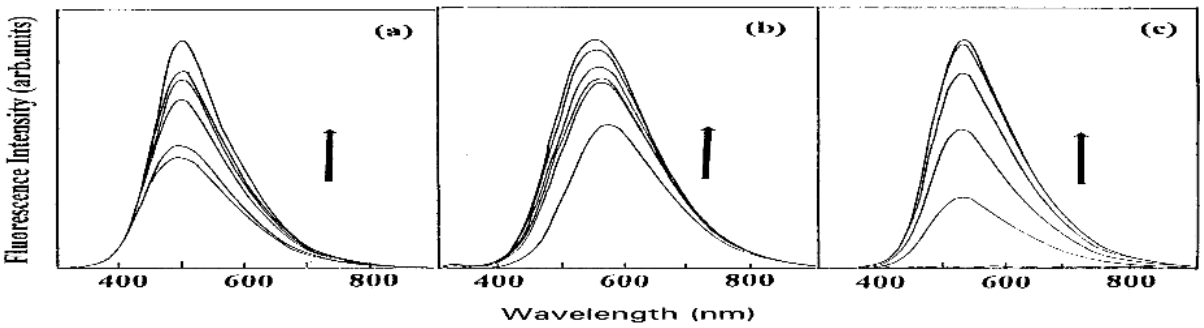

Emission enhancement observed for the three Ru(II) complexes (10 mM, buffer A) with increasing concentration of CT-DNA. (**a**) [Ru(phen)3]2+, (**b**)[Ru(phen)2(phen-dione)]2+ and (**c**) [Ru(phen)2(dppz)]2+. Maximum DNAconcentrations added (nucleotide phosphates) are 800, 800 and 100 mM for (a), (b)and (c), respectively.

TABLE.1 UV/Visible data.

$[Ru(phen)_3]^{2+}$	446 (4·28), 422 (4·25), 263 (5·07), 223 (4·93)
$[Ru(phen)_2(phen\text{- }dione)]^{2+}$	441 (4·29), 329 (4·51), 261 (5·11), 225 (4·92)
$[Ru(phen)_2(dppz)]^{2+}$	443 (4·33), 369 (4·39), 360 (4·33), 277 (5·12), 225 (5·02)

aSpectra were measured in CH3CN. Error limits: lmax, ± 2 nm; loge, ± 10%

TABLE.2 Results of absorption titration (*Kb*) and thermal denaturation (*Tm*) studies.

Complex	*Kb*, M^{-1} [a]	*Tm* °C [b]	σ*T*° [c]
[Ru(phen)3]2+	$7{\cdot}88 \times 10^3$	66	27
[Ru(phen)2(phen-dione)]2+	$2{\cdot}87 \times 10^3$	66	25
[Ru(phen)2(dppz)]2+	$>10^6$	67	26

aError limit: ± 10%
b[DNA nucleotide phosphate]/[drug] = 25; error limit ± 1°C and cError limit: ± 2°

Results and discussion:

Although a few among the investigated complexes have previously been spectrally characterized to their structure , this study has provided an opportunity to compare the spectroscopic and other physical properties of all the complexes by using data obtained under the similar set of experimental conditions. In addition, during the course of this study it was found necessary to compile and compare the physico-chemical characteristics of each investigated complex in order to choose the appropriate technique for probing the DNA interaction. Thus, we compare the physical and spectroscopic characteristics of the investigated Fe(III), Ni(II) and Ru(II) complexes before we discuss their DNA binding and photocleavage properties.

As far as the DNA interactions of these complexes are concerned, DNA binding by $[M(phen)_3]^{n+}$ (M = Fe(III) or Ru(II) and *n* = 2 or 3) and $[Ru(phen)_2(dppz)]^{2+}$ have been investigated in great detail by several groups and that of $[M(phen)_2(dppz)]^{n+}$ (M = Fe(III) or Ni(II) and *n* = 2 or 3) in lesser detail . However, relatively few studies seem to have been attempted to investigate the effect of variation of the metal ion and also the ligand on the ability to bind and photocleave DNA in mixed ligand complexes containing the

phenanthroline family of ligands. In the present study, we have endeavoured to compare the DNA binding and photocleavage properties of a series of $[M(phen)_2(LL)]^{2+}$ type complexes under similar experimental conditions.

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