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Biophysical and biochemical investigation on the binding of a manganese–cyanonitrosyl complex with DNA

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Abstract

The interaction of a cyanonitrosyl–manganate complex with DNA has been studied by u.v.–vis spectroscopy, circular dichroism, fluorescence and gel electrophoresis techniques. As the DNA 260 nm band remained insensitive to interaction, the interaction ratio was determined by monitoring the $CN^- \rightarrow Mn$ LMCT band observed at 222 nm, and the interaction ratio was found to be in the complex (D): DNA (P) = 1:0.30 from the spectrophotometric titration. The above-mentioned physical measurements indicate that the binding mode is not intercalative and the cyanonitrosyl system is a groove binder.

Introduction

During the end of the last decade, the biochemical role of nitric oxide, NO, was widely studied since it was found to be an essential component in many physiological processes. Biological processes mediated by NO include neurotransmission, blood pressure regulation and immunological responses. Dysfunction in NO metabolism has been associated with a number of disease states, such as epilepsy, arthritis, hypertension and septic shock [1–6]. In recent years, the interaction between transition metal complexes and DNA has been extensively studied [7] and utilized for developing novel chemotherapeutic and footprinting agents, and for gene manipulation in biotechnology and medicine. Binding studies of small molecules to DNA are important in the development of new therapeutic reagents and DNA molecular probes [8–11]. Transition metal complexes are employed mainly in this area because of their unique spectroscopic signature, which can be exploited for developing efficient probes for nucleic acid structure and conformation [12–13]. Interestingly, a large number of octahedral complexes of first row transition metal ions have been found to bind the grooves of DNA [14–15]. Metal nitrosyl complexes have also therapeutic value. Sodium nitroprusside, $Na_2[Fe(NO)(CN)_5] \cdot 2H_2O$, is used clinically to treat cardiovascular disorders and to lower blood pressure through release of NO. In this regard, the present project of investigating the interaction of DNA with Mn–NO complex has been taken up.

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Experimental

Materials and physical methods

All reagents were of A.R. or G.R. grade. The analytical grade solvents used for physico-chemical studies were further purified by the literature method [16] before use, wherever necessary. All experiments involving the interaction of the complex with DNA were carried out in BPES buffer in triple distilled water (all glass apparatus). A solution of calf-thymus DNA in the buffer gave a u.v. absorbance ratio at 260 and 280 nm of *ca.* 1.8–1.9, indicating that DNA was sufficiently free from protein [17]. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 M^{-1} cm^{-1}) at 260 nm [18].

Preparation of the complex

The Mn– cyanonitrosyl complex was prepared following the method reported earlier from this laboratory [19].

U.v.-spectral study

An appropriate amount of DNA (2–3 mg) was dissolved in BPES buffer (50 cm^3), kept overnight in the refrigerator, followed by addition of BPES to make up to the mark. The interaction ratio of the Mn–cyanonitrosyl complex with DNA (10^{-4} M) was determined by u.v. spectrophotometry. In the present work, DNA was allowed to interact with different concentrations of the Mn complex and incubated for 24 h at 37 °C, and the 260 nm DNA band was monitored thereafter. In all cases it was found that this band remained insensitive to all reaction conditions. So it was decided to monitor the

222 nm charge transfer band of the Mn–complex keeping the appropriate amount of DNA as a reference. This band modifies as a function of increasing the amount of metal complex, which ultimately became static at a certain molar ratio of complex:DNA (Figure 1). At this point the absorbance at λ_{222} was plotted against the molar ratio of the complex:DNA (Figure 2). The intercept of the curve defines the interaction ratio, which is 1:0.30 [complex:DNA].

Circular dichroism (c.d.) study

The c.d. spectra of the DNA solution (10^{-3} M) and DNA–Mn complex solution at different complex:DNA ratios of Mn–cyanonitrosyl complex, with the same concentration as that of the test solution, were recorded.

Gel electrophoresis study

Isolation of plasmid DNA

A pure culture of *E. Coli* was incubated at 37 °C for 24 h in a nutrient broth containing ampicillin. The broth was harvested after 24 h and centrifuged at 6000 × *g* for 15 min, decanted and the entire medium was drained. The pellet was resuspended in 10 cm^3 of GTE (glucose Tris–EDTA) buffer by vortexing. A 10 cm^3 solution of NaOH and SDS buffer were added and kept at room temperature for 5 min, and then 10 mg of chilled potassium acetate solution was added and mixed immediately, kept in the ice bath for 5 min, then centrifuged at 12,000 × *g* for 30 min at 4 °C. The pellet containing cell debris, genomic DNA *etc.* was discarded and the supernatant was transferred to a polypropylene tube. Then 0.8 cm^3 of *i* PrOH was added, mixed and was allowed to settle at room temperature for 5 min. The supernatant was discarded. The pellet was dissolved in 70% EtOH, and then spun again at 10,000 × *g* for 10 min. The pellet, which contained the plasmid DNA, was then vacuum dried and re-dissolved in Tris–EDTA (TE) buffer. This last step was repeated to ensure further purification. The homogeneity of plasmid DNA was checked by gel electrophoresis. Plasmid DNA was suspended in TE buffer for the gel electrophoresis study. Ten microliter of a DNA solution and various amounts of the Mn–cyanonitrosyl complex were added to it, then incubated at 37 °C for 2 h. The DNA complex and the solution of plasmid DNA (control) was loaded in 1% agarose gel with the addition of 10 μl of loading dye (Bromophenol blue). It was allowed to run for 2 h at 90 V.

Results and discussion

Electronic absorption studies

Electronic absorption spectroscopy is often employed to ascertain the binding of complexes with DNA. A complex bound to DNA through intercalation is characteristic of hypochromism and the red shift, due to intercalative mode involving a strong stacking interaction between an aromatic chromophore and the base pairs of DNA [20–22]. The absorption spectrum in the absence and presence of increasing amounts of DNA concentration is shown in the Figure 1. The 222 nm c.t. band of the complex is modified after interaction with DNA. The interaction ratio of complex (D): DNA (P) was found to be 1:0.30 from spectrophotometric titration (Figure 2). Since our complex does not contain any fused aromatic ring to facilitate intercalation, classical intercalative interaction is precluded. The hyperchromism of the Mn–cyanonitrosyl complex, on addition of calf-thymus DNA, implies that the binding mode is non-intercalative in nature. Similar hyperchromism has been observed for the Soret bands of certain porphyrins when interacted with DNA but which have not yet been clearly explained [23]. In order to compare quantitatively the binding strength, the intrinsic binding constant with calf-thymus DNA were obtained by monitoring the changes in absorbance at 222 nm with increasing

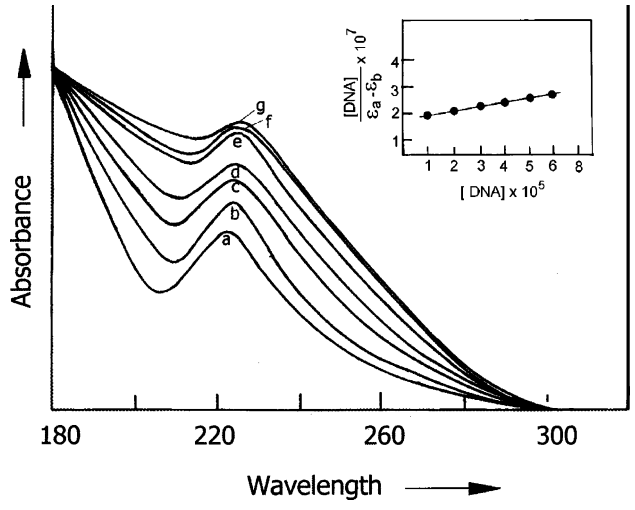

Fig. 1. Absorption spectra of the complex in the absence (a) and in the presence of increasing amounts of DNA (b, c, d, e, f, g) and a plot of the binding constant curve (inset).

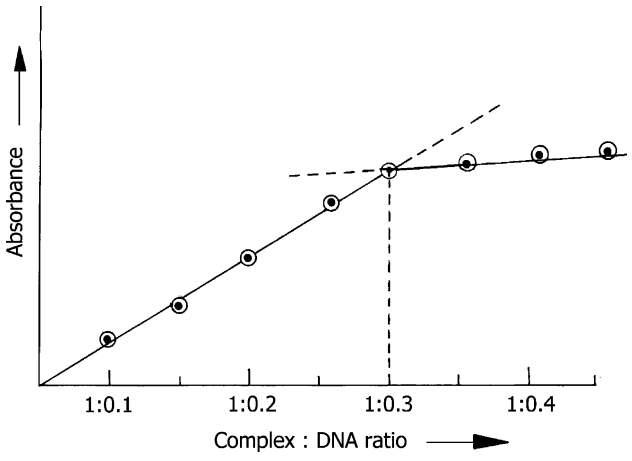

Fig. 2. Plot to determine the interaction ratio of the system.

concentration of DNA, by employing the equation shown below [24]:

$$\frac{[DNA]}{(\varepsilon_a - \varepsilon_b)} = \frac{[DNA]}{(\varepsilon_b - \varepsilon_f)} + \frac{1}{K_b(\varepsilon_b - \varepsilon_f)}$$

Here [DNA] is the concentration of DNA in base pairs, the apparent absorption coefficients ε_a, ε_f and ε_b correspond to A_{obsd}/[Mn], the extinction coefficient for the free manganese complex, and the extinction coefficient for the manganese complex is in the fully bound form, respectively. Plots of [DNA]/($\varepsilon_a - \varepsilon_b$) *versus* [DNA] gave a slope $1/(\varepsilon_b - \varepsilon_f)$ with intercept $1/K_b(\varepsilon_b - \varepsilon_f)$; K_b is the ratio of the slope to the intercept. The binding constant obtained ($K_b = 1.1 \times 10^2$) is not comparable to the classical intercalators like EB-DNA, (1.4×10^6 M^{-1}), in 25 mM Tris–HCl/40 mM NaCl buffer, pH = 7.9 [25]. This indicates the lower affinity of the complex towards DNA than the classical intercalators. This binding study also supports our proposition of the non-intercalative binding by the complex. Again, covalent binding of the bases of DNA is known to alter the absorption of DNA. As in the present case, no such change in the DNA 260 nm band is observed, the covalent binding of the bases of DNA is also ruled out giving a strong hint of associative groove binding (*vide* binding constant data).

Fluorescence spectroscopic studies

Ethidium bromide (EB) emits intense fluorescence in the presence of DNA due to its strong intercalation between the adjacent DNA base pairs. It has been previously reported that the enhanced fluorescence can be quenched by the addition of a second molecule [26]. The emission spectrum of EB bound to DNA, in the absence and presence of Mn–cyanonitrosyl complex, is given in Figure 3. The addition of EB to the DNA–metal complex aggregate registers a reduction in the fluorescence intensity of it, compared to the one where the complex is not added, which indicates that the complex inhibits the entry of EB into the base pairs.

Circular dichroism study

The results of the c.d. studies are presented in Figure 4. Pure DNA has a characteristic positive band at *ca.* 275 nm and a negative band *ca.* 245 nm. In the present study, when DNA was allowed to interact with the complex, these bands were modified. The band at 245 nm was reduced only to a hump, while the positive band was retained. To understand the conformational pattern c.d. spectrum of DNA-EB was also recorded. EB (a known DNA intercalator) as usual, enhanced the c.d. bands (Fig. 4), but when DNA was given to interact with the complex and then EB added, DNA did not show a spectrum characteristic of DNA-EB. The c.d. studies indicate that the complex modified DNA band, possibly because EB does not find any opportunity to enter into the base pairs of DNA. This can only happen when some extraneous molecules block the exterior sides of the DNA. Considering the above findings it is proposed that in the present case the complex is attached to the grooves of the DNA in such a way that EB does not find any way to enter the DNA base pairs.

Gel electrophoresis study

There has been considerable interest in DNA cleavage reactions that are activated by metal ions or metal complexes [27]. The cleavage reaction on plasmid DNA can be monitored by agarose gel electrophoresis. The result of the gel electrophoresis study is presented in Figure 5. In the gel electrophoregram four bands are

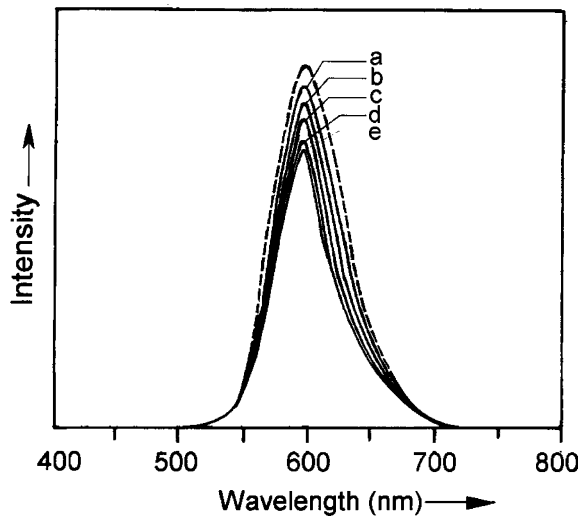

Fig. 3. Fluorescence spectra of EB bound to DNA in the absence (–) and presence of complex (–). [Complex]/[DNA] = 1:0.05 (a), 1:0.10 (b), 1:0.20 (c), 1:0.30(d), 1:0.45(e).

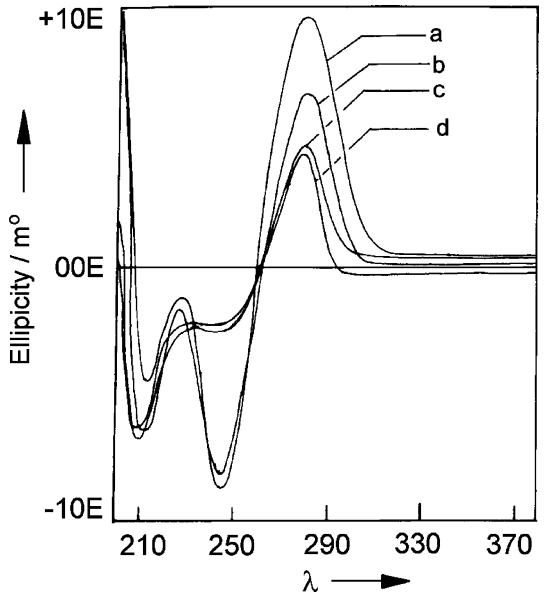

Fig 4. C.d. spectra: (a) DNA + EB solution. (b) DNA solution. (c) DNA + complex at the interaction ratio. (d) DNA + EB + complex solution at the interaction ratio.

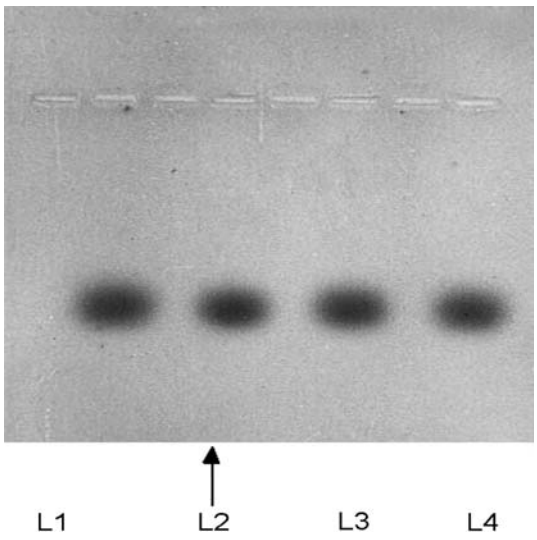

Fig. 5. Results of the gel electrophoresis experiment: lane 1 (L1) is complex: DNA (1:0.45), lane 2 (L2) is complex: DNA (1:0.30, which is at the interaction ratio, shown by↑), lane 3 (L3) is the complex: DNA (1:0.15), lane 4 (L4) is the control DNA.

observed, where lane 1 (L1) is complex:DNA (1:0.45), lane 2 (L2) is complex:DNA (1:0.30), lane 3 (L3) is complex:DNA (1:0.15) and lane 4 (L4) is the control DNA. The present study with agarose gel electrophoresis does not show any evidence of DNA cleavage, demonstrating clearly that the Mn–cyanonitrosyl complex does not induce any DNA cleavage under the experimental set up.

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