CHAPTER 6

SPECTROSCOPIC STUDIES ON THE INTERACTION OF CALF THYMUS DNA WITH ACRIDINEDIONE DERIVATIVES

6.1 INTRODUCTION

The basic structural elements of double helical DNA are vertically \( \pi-\pi \) stacked heteroaromatic bases, in plane hydrogen-bonded Watson-Crick base pairs having peripheral polar groups, and polyanionic phospho diester backbones. These moieties provide the sites of intercalation, H-bonding and electrostatic interaction respectively for DNA-binding proteins, antibiotics and synthetic molecules (Asakawa et al 1992). There are four stages of development of new synthetic DNA-binding molecule; design, synthesis, testing for sequence specificity and reanalysis of the design (Dervan 1986).

Three major aspects of the binding of drugs to DNA could be expected to influence their biological activity i) mode of interaction with the double helix ii) sequence specificity of binding and iii) kinetics of association/dissociation (Bourdouxhe-Housiaux et al 1996).

The concept of drug intercalation with DNA was originally proposed by Lerman (1961) which proved to be stimulant to our understanding of the molecular mechanism of action of many drugs, mutagens and carcinogens. The intercalation model provides first order explanations for such biological properties as inhibition of DNA-directed RNA synthesis and frameshift mutagenesis. Binding study of small molecules with DNA are important in
the designing of new and more efficient drugs targeted to DNA. In addition to it, various binding studies help to probe the DNA structure. Metallointercalators have been particularly useful in probing DNA structure and the intercalation process (Barton et al 1984).

Absorption spectroscopy is used to examine the thermodynamic properties associated with the interaction of the binding molecules with nucleic acids (Crenshaw et al 1995). Spectrophotometric and spectrofluorometric methods are well versed techniques for quantitative measurements of binding studies which provide useful information about the complex formation (Harris and Bashford 1988).

Since acridinedione compounds are expected to have interaction with DNA molecules, the spectroscopic studies of them would give some ideas on their interaction. Based on this, two of the compounds (TPDN and BADN-II), whose crystal structure has been presented in the earlier chapters have been used as model compounds for the spectroscopic studies. In this chapter, the spectroscopic studies of thiapyranedione (TPDN) and bis-acridinedione (BADN-II) to calf thymus DNA (ct-DNA) are described.

6.2 MATERIALS AND METHODS

The calf thymus DNA (ct-DNA) was purchased from Sigma-Aldrich Chemicals, USA and it was straightaway used for this study without further purification. Its concentration was determined spectrophotometrically by using extinction coefficient of 6600 dm³ mol⁻¹ cm⁻¹ at 260nm (Maiti and Chaudhuri 1981). Both the compounds TPDN and BADN-II are soluble in acetonitrile and the fresh solutions of TPDN and BADN-II were prepared using acetonitrile of spectroscopic grade.

Absorption spectra for TPDN were recorded using Hitachi A2000 spectrophotometer against an appropriately prepared reference sample in
1cm cuvette. The absorption spectra were obtained using 10mM phosphate buffers and 150mM NaCl - pH 7.2 (Fukui and Tanaka 1996) and acetonitrile. The DNA solution with acetonitrile was used to check the contribution of acetonitrile or any other peak in the DNA drug complex region. It revealed no significant peaks.

The absorption maxima for the sample TPDN was found to be 207nm in the UV region. At fixed concentration of TPDN (20μM) and varying the concentration of DNA (141.82μM, 212.72μM and 283.63μM), the absorption measurements were recorded. The spectra are shown in Figure 6.1. For TPDN, only absorption study has been carried out as there is no fluorescence observed for this compound.

Fluorescence measurements of BADN-II with ctDNA were obtained from Kontron SFM25 spectrofluorometer in 1cm cuvette. The absorption spectra for the compound with DNA were noted initially and fluorescence titration of DNA with BADN-II was performed with excitation at their maximum absorption wavelength and the emission spectra recorded upto 500nm. Fluorescence titrations were carried out for different concentrations of DNA (48.88μM, 122.22μM and 195.55μM) by keeping the concentration of BADN-II fixed (10μM).

The intrinsic binding constant (K) of TPDN with ct-DNA was calculated from the plot of \( \frac{D}{A_{ep}} \) versus D, where D is the concentration of DNA in base pairs.

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\Delta e_{ap} = [e_a - e_d] \quad \text{and} \quad \Delta e = [e_b - e_f].
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The apparent extinction coefficient, \( e_a \) is obtained by calculating \( \frac{A_{obs}}{[TPDN]} \), where \( A_{obs} \) is the intensity observed for particular DNA concentration. \( e_b \) and \( e_f \) correspond to the extinction coefficient of the bound form of TPDN and extinction coefficient of free TPDN respectively. The
intrinsic binding constant \( K (18.182 \times 10^4) \) is very high when compared with the reported value (Barton et al 1984; Pyle et al 1989).

6.3 RESULTS AND DISCUSSION

6.3.1 Spectrophotometric studies

The absorption maxima for the compound TPDN alone was found to be 207nm. The addition of DNA and increase in the concentration of DNA showed a strong decrease in the peak intensity (hypochromism). The absorption peak obtained for different concentrations of DNA are 141.82µM at 205.5nm, 212.72µM at 203.5nm and 283.63µM at 202.0nm. This spectral change, a blue shift of 5nm, showed that there was a possibility of complex formation of DNA and TPDN.

6.3.2 Spectrofluorometric studies

In spectrofluorometric titration, the fluorescence was observed for BADN-II at 458nm when excited at 388nm. The relative fluorescence (normalised) was 23.7 for the above sample. The addition of DNA and increasing the concentration of DNA showed no change in the excitation wavelength, but the fluorescence was enhanced for the minimum concentration of DNA with relative fluorescence 97.1. For the maximum concentration of DNA (195.55µM), the fluorescence was quenched as shown in Figure 6.2.

The fluorescence enhancement of BADN-II bound to ct-DNA may be due to shielding from water interactions involving transient H-bond formation. The fluorescence quenching may be due to excited state electron transfer from ct-DNA to BADN-II at the maximum concentration of DNA (Dogila et al 1993). Thus, there is a possibility of molecular aggregation without red shift or blue shift in the fluorescence.
Figure 6.1 Absorption spectra for fixed concentration of TPDN with different concentration of ct-DNA. (1) TPDN 20 uM, (2) 141.82 uM DNA + TPDN, (3) 212.72 uM DNA + TPDN, (4) 283.63 uM DNA + TPDN
Figure 6.2 Fluorescence spectra for fixed concentration of BADN-II with different concentration of ct-DNA. (1) BADN-II 10 μM, (2) 48.88 μM DNA + BADN-II, (3) 122.22 μM DNA + BADN-II, (4) 195.55 μM DNA + BADN-II