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Detailed spectroscopic investigations to reveal the nature of interaction of anionic porphyrin with calf thymus DNA

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ABSTRACT

The interaction between anionic form of *meso*-tetrakis(4-carboxyphenyl) porphyrin (TCPP) and calf thymus deoxyribonucleic acid (CT DNA) is investigated by measuring UV–vis absorption, steady-state fluorescence, steady-state fluorescence anisotropy, time-resolved fluorescence, resonance light scattering (RLS), FT-IR and circular dichroism (CD) spectra along with the help of atomic force microscopy (AFM) under Tris–Borate–EDTA (TBE) buffer solution of pH 8.3. The static mode of fluorescence quenching of porphyrin by calf thymus deoxyribonucleic acid indicates the formation of a ground-state complex. The formation of ground-state complex is a spontaneous molecular interaction procedure in which outside groove binding through hydrogen bond or van der Waals force plays a major role. For biomedical application this investigation is very important as here TCPP, i.e. the anionic porphyrin does not bring any changes in the original structure of the CT DNA to selectively cleaving the nucleic acid to destroy the cancer or tumor cells whereas cationic porphyrin makes change in the protein structure significantly during the same process.

1. Introduction

The interaction of small molecules with DNA continues to be an extremely important area of research, in the sense of both fundamental and practical point of view, as the molecular recognition of DNA is of fundamental importance to life. DNA is an important drug target, in particular in the treatment of cancer, where many compounds that bind covalently and/or non-covalently to DNA, or damage DNA, are used. Interaction of porphyrins and metalloporphyrins with DNA has a considerable interest due to their medical applications. The ability of porphyrin to selectively cleave nucleic acid has made the porphyrins widely used as a structural probe of DNA [1]. Also, the special properties of porphyrins and metalloporphyrins, e.g. high absorbance, relatively high quantum yields of fluorescence and triplet state or paramagnetism of some metal complexes lead the use of porphyrin in medicine, as active compounds in radiological [2,3], and magnetic resonance imaging [4,5] of cancer detection. Certain porphyrins are photosensitizers and can be useful in the photodynamic therapy of cancer [6–9] and act as inhibitor of HIV-I, the virus responsible for AIDS [10,11].

Three major binding modes have been proposed for the binding of porphyrin with DNA: (a) intercalation in which porphyrins intercalate into the base pairs of nucleic acids, (b) outside groove binding which involves hydrogen bonding or van der Waals interaction with the nucleic acid bases in the deep major groove or the shallow minor groove of the DNA helix and (c) outside binding with self-stacking involving porphyrins are stacked along the DNA helix. Intercalators have been widely used as antitumor, antineoplastic, antimalarial, antibiotic agents [12]. Like intercalators, groove binders have proven clinical utility as anti-cancer and antibacterial agents [12]. A great deal of works [13–19] have been studied on the interactions of cationic porphyrins with DNA as the formation of porphyrin–DNA complexes, one of the most important steps in effective therapeutic treatment of tumor activity, is known to be facilitated by the electrostatic attractions between the periphery of cationic porphyrins and the anionic phosphate backbone of DNA. Actually, cationic porphyrins are considered as double functional compounds that strongly bind to DNA and photodynamically modify the target site of a DNA molecule by a mechanism similar to that of anti-cancer antibiotics such as bleomycin and daunomycin based on the DNA cleavage [1,20,21].

On the other hand, the interaction of anionic molecules with DNA has not been as well studied [22]. In this paper we have investigated the interaction of an anionic porphyrin with calf thymus DNA (CT DNA). We have used here the anionic form to examine whether this negatively charged porphyrin could form porphyrin–DNA complex which is essential for designing anticancer drugs in biotechnology.

In the present investigation we have studied the interaction of an anionic porphyrin, e.g. *meso*-tetrakis(4-carboxyphenyl)

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porphyrin (TCPP) with CT DNA by measuring UV–vis, FT-IR absorption, steady-state and time-resolved fluorescence, fluorescence anisotropy, resonance light scattering (RLS) spectra. The studies were also made by using atomic force microscopy (AFM) and circular dichroism (CD) techniques under TBE buffer solution (pH 8.3) to understand the mode of the binding of porphyrin to DNA. This type of study will be effective to design new types of agents that can target DNA as part of their therapeutic action.

2. Materials and methods

2.1. Materials

The samples TCPP purchased from Aldrich (Fig. 1) and CT DNA supplied by Sisco Research Laboratories Pvt. Ltd. (SRL), India, are used as obtained.

2.2. Other chemicals used

The solvent used in these experiments is TBE buffer (pH ~ 8.3). The buffer Tris was purchased from Merck (German), boric acid and ethylenediaminetetraacetic acid (EDTA) used for preparing the buffer are of analytical purity. The solutions were prepared in TBE buffer solution (pH 8.3) using autoclave water to achieve anionic form of TCPP.

2.3. Methods

Steady-state UV–vis absorption, fluorescence emission spectra, steady-state fluorescence anisotropy and RLS spectra of dilute solutions (10^{-4}–10^{-6} M) of the samples were recorded at ambient temperature (296 K) using 1 cm path length rectangular quartz cells by means of an UV–vis absorption spectrophotometer (Shimadzu UV–VIS 2101PC) and F-4500 fluorescence spectrophotometer (Hitachi), respectively. Fluorescence lifetime measurements were carried out by the time-correlated single photon counting (TCSPC) method using HORIBA JOBIN YVON FLUOROCUBE. The excitation wavelength used was 405 nm (FWHM ~ 172 ps). The quality of fit was assessed over the entire decay, including the rising edge, and tested with a plot of weighted residuals and other statistical parameters, e.g. the reduced χ^2 and the Durbin–Watson (DW) parameters. The steady-state polarized emission spectra are measured by using UV–vis polarizer accessories including UV Linear Dichroic polarizer, wavelength range: 230–770 nm, purchased from Oriel Instruments, USA, with help of a Hitachi F-4500 fluorescence spectrophotometer equipped with a 150 W xenon lamp. While measuring the fluorescence anisotropy (r), the following Eqs. (1) and (2) are employed. The resolution of the spectrophotometer is about 1 nm and the respond time is approximately 4 ms.

$$r = \frac{I_{EE} - GI_{EB}}{I_{EE} + 2GI_{EB}} \quad (1)$$

where G is an instrumental factor (polarization characteristic of the photometric system) and is measured by using the relation

$$G = \frac{I_{BE}}{I_{BB}} \quad (2)$$

where I_{EE} and I_{EB} are the intensities of parallel and perpendicular polarized emission with vertically polarized excitation and I_{BB} and I_{BE} are the intensities of horizontally and vertically polarized emission when excited with horizontally polarized light. I_{BE}/I_{BB} defines the instrumental correction factor G (polarization characteristic of the photometric system). This correction is made for any change in the sensitivity of the emission channel for the vertically and horizontally polarized components. All the experiments are performed at ambient temperature (298 K) with air equilibrated solutions. Throughout the experiment, pH of the medium was kept constant at 8.3 using TBE buffer.

FT-IR measurements were carried out in a Perkin Elmer Spectrum 100 FT-IR spectrometer using a 0.1 mm CaF_2 cell.

AFM images of TCPP and CT DNA adsorbed on TCPP were obtained with a multimode AFM (Vecco Metrology, Autoprobe diCP-II, Model No. AP 0100) at ambient temperature using silicone probes (RTESPA-M,Vecco, Santa Barbara, CA) in tapping (NC-AFM) mode. Long tips (aspect ratio 4:1) cantilever (material: 1–10 Ω cm Phosphorous (n) doped Si) with spring constants ranging from 20 to 80 N/m and resonance frequencies of 245–285 kHz were used to image the surface morphology of the biomass. Initially the scanning area was 10 μm × 10 μm and subsequently reduced to isolate the individual cells. Offline section analysis of each image was performed to obtain information on the sample width and height. Proscan Image Processing Programme software provided by the manufacturer was used to measure the roughness of the samples. Surface roughness (R_{rms}) was measured from the following equation

$$R_{rms} = \sqrt{\frac{\sum(Z_i - Z_{avg})2}{Np}}$$

where Z_i is the current Z value, Z_{avg} is the average of the Z values within the given area and Np is the number of point within the given area.

CD spectra have been recorded by JASCO, CD Spectrometer; model J-815-150S using a 0.1 cm path length quartz cell in a wavelength range between 300 and 450 nm.

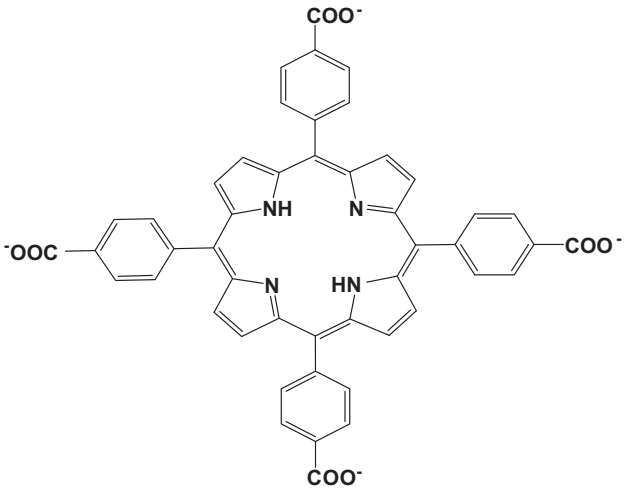

Fig. 1. Anionic form of TCPP.

3. Results

3.1. Steady-state UV–vis absorption studies

One of the most common techniques in DNA-binding studies of porphyrin compounds is electronic absorption spectroscopy. The absorption spectra of TCPP in presence of CT DNA in TBE buffer are shown in Fig. 2a. The sharp peak at ~415 nm corresponds to the characteristic absorption of the porphyrin-Soret band of TCPP whereas the smaller peaks between 500 and 700 nm are assigned as Q bands. From the Fig. 2a it is seen that with each addition of CT DNA to TCPP solution, the entire absorption spectrum