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Interaction of aluminum phthalocyanine with aziridinyl quinone in biomimicking micellar microenvironment for the application in photodynamic therapy: Effect of micellar hydration

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ABSTRACT

Aluminum phthalocyanine tetrasulphonate ($AlPcS_4$) is a well characterized water soluble phthalocyanine dye having structural similarities with porphyrin and has immense applications as a photosensitizer in photodynamic therapy (PDT). The present article embodies the exploration of physicochemical properties of PDT active $AlPcS_4$ and its interaction with DNA alkylating quinone in biomimicking micellar microenvironments to overcome the limitation of PDT that is caused by the hypoxic nature of solid tumor. UV–vis absorption, steady state emission and time resolved fluorescence spectroscopy reveal that the $AlPcS_4$ does not undergo considerable interaction with anionic SDS as well as uncharged Triton X-100 micelle, whereas in case of the series of a cationic surfactant (DTAB, TTAB, CTAB) it shows a significant columbic attraction toward the positively charged head group of the surfactant molecule. In premicellar concentration, surfactant induced aggregation of the probe molecule is observed, which subsequently disaggregates into its monomeric form above critical micellar concentration. The anionic dye localizes itself in the Stern layer of cationic micelles. The escalation of the fluorescence anisotropy value (r) with increase in the surfactant concentration is explained by the rise in compactness around the probe with increasing chain length of the cationic surfactant. However, the compactness has a reverse effect on the extent of water penetration or micellar hydration, which in turn decreases the polarity of microenvironment in the Stern layer. The fully micellized PDT active $AlPcS_4$ experiences stronger interaction with DNA alkylating quinone in confined medium as compared to the aqueous solution and the above said interaction intensifies with decrease in the micellar hydration. This spectroscopic research described herein may provide relevant addition to the usefulness of this bioactive dye–quinone system for the application in photodynamic therapy.

1. Introduction

Phthalocyanine has attracted a considerable attention as a second generation photosensitizer in photodynamic therapy (PDT) [1,2]. These dyes are widely studied for their rich photophysical behavior and application in photochemistry [3–6]. Incorporation of sulphonate group in phthalocyanine increases its water solubility and hence they are found to be more useful in PDT. However, sulphonated phthalocyanines are prone to form aggregates in water, which are less fluorescent and show lower quantum yield and hence less photosensitizing activity [4].

Photodynamic therapy is usually considered as a type of photochemotherapy, which requires red laser light, a photosensitizing agent, and molecular oxygen [1,7,8]. Typically when a photosensitizer is excited by red laser normally it returns to the ground state via triplet excited state by releasing its triplet energy. This triplet energy is concurrently absorbed by triplet oxygen and converts itself into singlet oxygen which eventually kills the tumor cells [9]. Production of singlet oxygen is generally referred as pathway II, the most important process of PDT as it directly involves killing of tumor cells. On the other hand, in type I mechanism,the triplet photosensitizer reacts with the substrate and forms free radicals which leads to cytotoxic events in PDT, especially in hypoxic environments [8,10].

Solid tumor are often hypoxic in nature, hence the direct killing of tumor cell by type II mechanism is limited due to hypoxia [11]. In this scenario biologically reducible drugs are potentially used against solid tumors [12]. The bioreductive compounds which have

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shown most extensive clinical applications are the aziridinyl quinones [12,13]. They have significant activity against many tumor models and are under clinical use for several years [13]. These compounds contain a reducible quinone moiety and an aziridinyl group which can form covalent bonds with variety of cellular components including DNA. However, the reduction of the quinone moiety is essential for the activation as well for the alkylating activity of these agents. Once activated, these compounds may form the two-electron reduced species semiquinone and hydroquinone, and interestingly both of them are reported as DNA-alkylating species [12].

Thus, a pertinent question is the mode of reduction with lesser side effects, and in this context remembering the hypoxic nature of solid tumor, Alegria et al. [1] have reported the photosensitized reduction and subsequent DNA alkylation of aziridinyl quinones in recent past. Porphyrins, chlorin, phthalocyanine have been used as photosensitizer and diaziridinyl benzoquinones have been used as reducible substrate for this purpose. According to this hypothesis, since these red-light-absorbing PDT dyes are able to photo-reduce oxygen, these should also be able to photo-reduce quinone molecules, which are having nearly equal or more positive redox potentials than oxygen, in anoxic/hypoxic cells. Once the DNA alkylating quinone is activated by reduction, it may lead to in DNA alkylation with the consequent cell death [1,12,14].

In order to support the above hypothesis, a complete understanding of the physicochemical behavior of the PDT active phthalocyanine and porphyrins in absence and presence of alkylating quinone in the cellular environment is essential, because the therapeutic efficacy of the tetrapyrolic pigments may depend on its aggregation, aggregation–disaggregation behavior, localization and interaction pattern (in absence/presence of alkylating quinone) in the cellular environment. In this context the interaction of $AlPcS_4$ is experimented with the DNA alkylating aziridinyl quinone in biomimicking micellar systems. Micelles are often used as membrane biomimicking agent [15,16]. The importance of membranes in biological systems lies in their capacity to provide a matrix for arranging the reaction sequentially for efficient interaction. Substrate molecules may be sequestered and organized in the micellar interior or on the micellar boundary [17,18]. The penetration of PDT probe in micellar media may modify its photophysical behavior because micelle produces nonpolar–polar interfaces where absorption, emission properties of dyes becomes enhanced or quenched [19,20].

In this article we have attempted the encapsulation of the PDT active $AlPcS_4$ along with DNA alkylating aziridinyl quinone in single organized assembly that can bring them together. This may result in increased possibility of the association between these two or it may alter their interaction pattern [21]. Charges on micelle may also influence pronounced effect on such types of interactions. $AlPcS_4$ is highly effective PDT dye and several studies have been investigated using $AlPcS_4$ as probe [1–6,22], but to the best of our knowledge the interaction of this PDT active $AlPcS_4$ with DNA alkylating aziridinyl quinone in biomimicking micellar microenvironment is not reported so far. Hence, in the present article, a conspicuous attempt has been made at first to demonstrate the physicochemical studies of $AlPcS_4$ in different cationic micelles of varying chain length and then to monitor its interaction with DNA alkylating quinone; 2,5-diclorodiaziridinyl-1,4-benzoquinone (AZDCIQ) in aqueous as well as in above three micellar microenvironment using UV–vis absorbance, steady state fluorescence, fluorescence anisotropy and time resolved fluorescence study. This will in turn epitomize the nature and strength of interaction between $AlPcS_4$ and alkylating quinone AZDCIQ inside cell like environment. Thus this may be a relevant addition to use the water soluble PDT dye and reducible substrate together in anoxic/hypoxic environments which may open up new avenues for PDT treatment.

2. Materials and methods

2.1. Chemicals

Aluminum phthalocyanine tetrasulphonate ($AlPcS_4$) is procured from Frontier Scientific, USA. The alkylating quinone 2,5-dichloro-diaziridinyl-1,4-benzoquinone (AZDCIQ) and cetyltrimethylammonium bromide (CTAB), Triton X-100 (TX-100) are obtained from Sigma–Aldrich Chemicals, USA. Dodecyltrimethylammonium bromide (DTAB) and tetradecyltrimethylammonium bromide (TTAB), sodium dodecylsulphate (SDS) are obtained from Spectrochem, India and Alfa Aesar, England, respectively. All the chemicals are used as received.

2.2. Instrumentation

The steady state absorption and emission are recorded at 298 K using Jasco V-630 spectrophotometer equipped with peltier accessories and Jasco FP-8300 spectrofluorometer equipped with temperature controller unit respectively. All the measurements are carried out using 1.0 cm quartz cell and an external slit width of 2.5 nm. The steady state fluorescence anisotropy is also performed using Jasco FP-8300 spectrofluorometer along with polarizer accessories. For the anisotropy measurement the excitation and emission bandwidths are set at 2.5 nm each. The steady state anisotropy is calculated by the following equation:

$$r = (I_{VV} - GI_{VH})/(I_{VV} + 2GI_{VH})$$

where, I_{VH} and I_{VV} are the intensities obtained from the excitation polarizer oriented vertically and the emission polarizer oriented in horizontal and vertical directions, respectively. The factor G is defined as I_{HV}/I_{HH}. Fluorescence lifetime measurement are performed using PTI Time Resolved Spectrofluorimeter PicoMaster (PTI, HORIBA, USA), laser diode (405 nm) as a light source and time correlated single photon counting (TCSPC) technique. The data are analyzed using the *FelixGX* 4.1.2 decay analysis software. The exponential fittings are authenticated by reduced χ^2 criterion and the randomness of the fitted function of the raw data. The average fluorescence lifetime $<\tau>$ for biexponential decay of the dye are calculated using decay times (τ_i) and the relative contribution of components (α_i) using the following equation.

$$<\tau> = \frac{\sum \tau_i \alpha_i}{\sum \alpha_i}$$

All the above experiments are repeated several times in order to obtain reproducible results.

2.3. Sample preparation

Millipore water is used for preparing all the solutions and the pH is adjusted as required. Oxygen is excluded from the samples by keeping a positive pressure of argon during all the experiments. Deoxygenation is stopped while recording the absorption and emission spectra. The stock solution of $AlPcS_4$ (0.2 mM) is prepared in millipore water and small aliquot are used during experimentation. AZDCIQ stock is prepared by adding it in millipore water followed by warming, cooling and filtration. The different surfactant solutions are prepared by dissolving required amounts of SDS, TX-100, DTAB, TTAB, or CTAB in millipore water at pH 7.4 (Scheme 1).