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Fluorescence quenching effect of guanine interacting with water-soluble cationic porphyrin

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ABSTRACT

The process of association between 5,10,15,20-tetrakis[4-(trimethylammonio)phenyl]-21*H*,23*H*-porphine tetra-*p*-tosylate (H_2TTMePP) and guanine has been studied both in NaOH solution and TRIS buffer analysing its absorption and steady-state fluorescence spectra. The fluorescence quenching effect observed during interactions porphyrin-guanine points at the fractional accessibility of the fluorophore for the quencher. The association and fluorescence quenching constants are of the order of magnitude of 10^5 mol^{-1}. The fluorescence lifetimes and the quantum yields of the porphyrin monoanion form were established. The results demonstrate that guanine can interact with H_2TTMePP at basic pH and through formation of stacking complexes is able to quench its ability to emission.

1. Introduction

Water-soluble porphyrins are the compounds with the specific spectroscopic and redox properties, as well as the ability to transfer electrons, very sensitive to the subtle changes of pH, porphyrins and ligands concentration or the form of complexing with metal ions proceeding in a reaction environment. The representatives of the class of water-soluble porphyrins are the cationic porphyrins, which have the great ability to interact with DNA chain and its particular elements [1–5]. Spectral analysis of interactions between the porphyrin compounds and purine or pyrimidine bases plays a pivotal role in better understanding of organisms functioning, what can be useful from a therapeutic point of view, for example to identify ligands with potential anticancer activity (that is molecules that stabilize G-quadruplexes in DNA) [1,6], in studies of telomerase inhibitors [1,6,7] and photodynamic diagnosis and therapy of cancer (PDT) [8–12].

One of the nucleic bases interacting with water-soluble cationic porphyrins is guanine (2-amino-6-hydroxypurine). The bicyclic planar molecule of guanine can bind in DNA to cytosine through three hydrogen bonds, where the group at C-6 acts as the hydrogen bond acceptor, while the group at N-1 and the amino group at C-2 act as the hydrogen bond donor. Despite the unquestionably important role in metabolism of nucleic acids, in case of an inborn error, guanine metabolism can be sometimes associated with uric acid overproduction, causing among other things arthritis, gout or lithiasis [13]. Extracted from scales of some fishes, guanine can play as well a role of a natural pearl essence with a low specific gravity (1.6 g/cm^3) and high refraction index (1.85) [14], which in spite of a high production costs is applied in the cosmetics industry as an additive to various products (e.g., shampoos, nail polishes, eye shadows), providing a pearly iridescent effect and bright skin tone (Uguisu mask [15]).

The studies described in this paper were focused on the spectroscopic analysis of binding interactions between biologically important molecules. Since guanine is not soluble in neutral solutions, the primary objective of presented research was to determine the mechanism of guanine–water-soluble cationic porphyrin 5,10,15,20-tetrakis[4-(trimethylammonio)phenyl]porphine (H_2TTMePP) interactions in basic microenvironment (Fig. 1). Increasing pH value of the reaction environment leads to the oxidation of the nucleic bases. Among the all nucleic bases, guanine is the easiest target in oxidation process because of its high electronic density and consequently its lowest redox potential, what renders this compound especially useful to study the interactions with cationic porphyrin systems [16]. In previous work the method of absorption spectra and fluorescence quenching analysis proved to be a powerful tool to described the conformational changes of porphyrins during their stacking interactions with cyclic organic compounds, such as nucleic bases [16] and caffeine [17], therefore absorption, steady-state fluorescence, as well as fluorescence lifetimes and quantum yields measurements were considered during study of guanine—porphyrin system. The second objective was to answer the question if the choice between TRIS buffer or NaOH solution for measurements of described system registered at pH > 12 causes a noticeable difference in obtained results.

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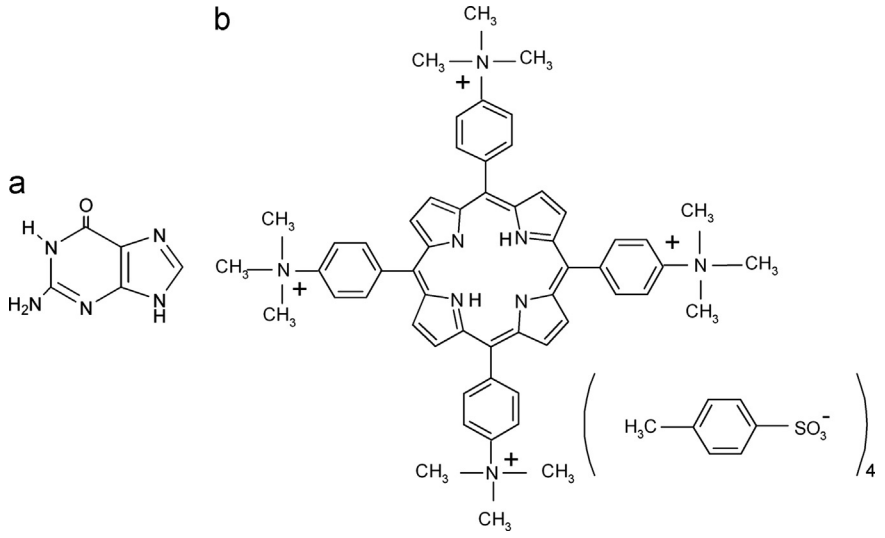

Fig. 1. The molecular structures of (a) guanine (2-amino-6-hydroxypurine), (b) H_2TTMePP (5,10,15,20-tetrakis[4-(trimethylammonio)phenyl]-21*H*,23*H*-porphine).

A great affinity of cationic free-base porphyrins to react with nucleic building blocks is presented in literature using primarily the other porphyrin compound—5,10,15,20-tetrakis(1-methyl-4-pyridyl)-21*H*,23*H*-porphine tetra-*p*-tosylate (H_2TMePyP), and its interactions with DNA or chosen mononucleotides [4]. The studies of guanine–porphyrin interactions are not popular (because of poor water solubility of guanine), therefore the experiments conducted in extreme conditions of pH could enrich the knowledge concerning the porphyrin fluorescence decay kinetics [2,4,18], designing of new classes of artificial receptors [19–24] or monitoring of the porphyrin—toxic substances interactions [25] and guanine metabolism [13], as well as environmental and sanitary parameters [19,21,26].

2. Experimental

2.1. Reagents

Guanine (2-amino-6-hydroxypurine), α,α,α-tris-(hydroxymethyl)-methylamin (TRIS) and 5,10,15,20-tetrakis[4-(trimethylammonio) phenyl]-21*H*,23*H*-porphine tetra-*p*-tosylate (H_2TTMePP, lg ε=5.59, 413 nm) were purchased in ALDRICH and used without any additional purification.

2.2. Measurements

The titration experiments were carried out using a 10^{-3} mol dm^{-3} stock solution of ligand (guanine) dissolved in 0.025 mol dm^{-3} TRIS (pH=12.5) or 0.01 mol dm^{-3} NaOH (pH=12.5) to improve the guanine solubility in water. TRIS buffer was already chosen before [16], therefore the measurements were continuing using the same buffer to make a proper comparison. The porphyrin solution was freshly prepared in TRIS buffer or NaOH solution at the concentration range about 10^{-7} mol dm^{-3}, to set the starting solution with the porphyrin absorbance value equals approximately 0.1 (to avoid the concentration fluorescence quenching effect on emission spectra of porphyrins [27,28]). The initial volume of the porphyrin solution used was 2 cm^3. The volumes of the stock guanine solution added at each step during titration of the porphyrin were as follows: 0, 0.005, 0.005, 0.005, 0.01, 0.01, 0.02, 0.03, 0.05, 0.1, 0.2, 0.3 and 0.3 cm^3 (final volume of stock guanine solution was 1.035 cm^3; final volume of solution in a cell was 3.035 cm^3). The concentration of H_2TTMePP during reaction with guanine dissolved in TRIS and NaOH was changing in the range 2.40–1.59 $\times 10^{-7}$ mol dm^{-3}. The final concentration of guanine in the mixture was 3.36×10^{-4} mol dm^{-3}.

Absorption spectra were recorded on JASCO V-660 spectrophotometer, using 1 cm Hellma quartz cells at the temperature of 21 °C. Photometric accuracy of the spectrophotometer used was equal ± 0.002AU. Steady-state fluorescence spectra, fluorescence lifetimes and quantum yields were recorded at room temperature on a UV–vis–NIR QuantaMaster™ spectrofluorometer (Photon Technology International) equipped with a continuous 75 W/450 W Xe-arc lamp as the light source. The excitation wavelength was 421 nm. The spectra were corrected with respect to the source and detector. The database program Sigma Plot (version 9.0) (Jandel Corp.) was used in the manipulation and plotting of the data.

2.3. Calculation of the association and fluorescence quenching constants for porphyrin–guanine systems

The calculations of association constant K_{AC} of porphyrin–ligand (guanine) complex were done taking into consideration the 1:1 model of complex formation, using the equation based on Bjerrum function modified by Beck [29] and the non-linear fitting procedure based on Marquardt–Levenberg algorithm (program Sigma Plot), as it was described previously [16,17].

The steady-state fluorescence quenching data were initially analysed based on the classic Stern–Volmer equation [30]:

$$\frac{F_0}{F} = 1 + K_{SV}[Q] \tag{1}$$

where F_0 and F are the fluorescence intensities in the absence and presence of quencher, respectively; [Q] is the concentration of quencher, K_{SV} is the Stern–Volmer quenching constant. Thereafter the modified form of the classic Stern–Volmer relation (the Lehrer equation) was used, on the understanding that the sample contained more than one fluorophore population with different accessibilities [31]:

$$\frac{F_0}{F_0 - F} = \frac{1}{\alpha K_{SV}[Q]} + \frac{1}{\alpha} \tag{2}$$

where α is the fraction of the accessible fluorophore population.

Employing the obtained results a number of binding sites was calculated

$$\log \frac{F_0 - F}{F} = \log K_{AC} + n \log [Q] \tag{3}$$