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Biophysical characterization of the interaction of human albumin with an anionic porphyrin

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ABSTRACT

The manuscript describes the characterization of the interaction between *meso*-tetrakis(*p*-sulfonatophenyl)porphyrin (TSPP) and human serum albumin (HSA). TSPP is a candidate for the photosensitization of structural and functional changes in proteins while HSA provides both an excellent protein model and binding and functional characteristics that could be explored in future applications of the approach. A combination of optical spectroscopic techniques (e.g., fluorescence spectroscopy, fluorescence lifetime, circular dichroism, etc.) and computational docking simulations were applied to better characterize the TSPP/HSA interaction. Recent advances have revealed that the complex formed by TSPP and HSA has become potentially relevant to biomedical applications, biomaterials research and protein photosensitized engineering. The study has determined a likely location of the binding site that places TSPP at a site that overlaps partially with the low affinity site of ibuprofen and places one of the SO_3^- groups of the ligand in proximity of the Trp214 residue in HSA. The characterization will enable future studies aimed at photosensitizing non-native functions of HSA for biomedical and biomaterial applications.

1. Introduction

The importance of porphyrins in living organisms is underscored by their ubiquitous biochemical functions and by their potential biomedical and biotechnological applications [1,2]. Heme, for instance, occupies a central role in a myriad of biological functions [2]. The combination of the natural occurrence of many porphyrin-like molecules and their photophysical properties (e.g., relatively large yields for intersystem crossing to the triplet state or the potential for photoinduced electron transfer [3,4]) has created widespread interest that includes basic photophysical and photochemical characterizations [5], self-assembly [6,7], and biomedical applications such as their use as a photosensitizers in the phototherapy and photodetection of cancer and other abnormal tissues [8–10]. The nuance of the photosensitization of porphyrins in biomedical applications has recently highlighted the role of the direct interaction between porphyrins and protein targets [11]. Many naturally occurring porphyrins often present the significant challenge of poor aqueous solubility and their consequent tendency to form a very polydispersed family of aggregates [13] which, despite having insignificant photochemical characteristics [13], greatly affect the spectroscopic characterizations. A possible circumvention of the problem is provided by cationic and anionic porphyrins. These are not-naturally occurring, but provide the advantage of easy solubility in aqueous solution. Among them is the anionic tetra-meso-phenylsulfonato-porphyrin (TSPP). At larger concentrations ($> 10^{-4}$ M) and lower pH (< 5) this porphyrin is also characterized by interesting self-assembly properties [12,14–16]. However at μM concentration and pH > 5.5 TSPP is substantially monomeric in aqueous solution [17]. Anionic (and cationic) porphyrins are expected to interact with proteins through different electrostatic mechanisms and are expected to dock at different locations of the protein [15] compared to heme or other hydrophobic tetrapyrroles.

As mentioned above, the focus of our research is to employ photosensitizing ligands to mediate changes in the structure and function of proteins. Our group has demonstrated that low-irradiance optical excitation of TSPP, non-covalently docked to proteins, causes conformational changes of some polypeptide [12,16]. These initial results prompted us to investigate the effects on proteins that are more relevant to biophysical and biomedical applications. One of these is represented by human serum albumin (HSA) which is the major carrier of exogenous and endogenous products in the bloodstream, including heme and other porphyrin products [18,19]. Its hypothesized role in the elimination of heme products, led to the

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discovery and characterization of a binding site for hemin (the Fe^{3+} chelate of heme), as well as other tetrapyrroles [20,21] and has produced much interest in the physiological and non-physiological properties of heme-HSA complexes [22,23]. The heme binding pocket has been experimentally identified in the primary cleft of subdomain IB of HSA and is defined by three basic residues at the pocket entrance and a Tyr residue (Tyr161) which coordinates with the metal at the center of the porphyrins ring [20]. In comparison with heme and other protoporphyrins, the size and charges on TSPP suggest potentially different mechanisms and sites of interaction. We have therefore investigated the non-covalent docking of TSPP to HSA with the goal of estimating the location of its binding site. Since this porphyrin has shown to be more effective at producing conformational changes of proteins [14,24], it holds the potential to prompt photosensitized changes in a biomedically crucial protein such as HSA. The possibility to trigger conformational changes using the combination of laser irradiation of a porphyrin ligand, could enable us to artificially enhance or modify the binding properties of HSA. This could have repercussions in future developments of the use of porphyrin/albumin complexes as an artificial enzyme [25], or a substitute for hemoglobin or myoglobin [26].

2. Materials and methods

2.1. Chemicals

Globulin-free HSA (A3782), warfarin (A2250), ibuprofen (I4883) and hemin (51,280) were purchased from Sigma-Aldrich (St. Louis, MO). Spectroscopic grade DMSO was also purchased from Sigma-Aldrich (St. Louis, MO) while TSPP was obtained from Frontier Scientific (Logan, UT). All chemicals were used without further purification.

2.2. Sample preparation

Concentrated stock solutions of the mono-dispersed TSSP were prepared at least 24 h prior to the experiments by dissolving the solid porphyrin in deionized water (DI). The stock solutions were subsequently kept in the dark at room temperature and were stable for up to two weeks. Before each experiment the stock solution was diluted in phosphate buffer at physiological pH, to obtain a final porphyrin concentration in the 2–8 μM range. The concentration was determined spectroscopically using the Beer-Lambert relationship [27] ($OD_{\lambda}=\varepsilon_{\lambda} cl$) with $\epsilon_{413}=510{,}000\ M^{-1}\ cm^{-1}$ for TSPP [28].

Stock solutions of HSA were prepared fresh before each experiment by dissolving the lyophilized protein in phosphate buffer at pH 7.4. Buffers were prepared at least 24 h in advance by dissolving a saline tablet (P4417, Sigma-Aldrich, St. Louis, MO) in 200 mL of DI water ($\Omega > 18\ \Omega$). All buffers were kept at 6 °C, and only the volume necessary for each experiment was withdrawn and allowed to equilibrate at room temperature before the start of the measurements. Buffers were discarded and made fresh every 7 days.

2.2.1. Instrumentation and methods

2.2.1.1. Steady state measurements. Absorption spectra were recorded using a dual beam Evolution 300 spectrophotometer (Thermo Fisher Scientific, Waltham, MA) at 1.5 nm resolution and 240 nm/min sampling speed. Steady state fluorescence spectra were recorded with an Aminco Bowman-2 double monochromator fluorimeter (Thermo Fisher Scientific, Waltham, MA) at 1 nm/s with a 4 nm bandwidth in excitation and emission. All spectra were corrected for the spectral response of the instrument as well as absorption and inner filter effects (Eq. (2)a and b).

Circular Dichroism spectra (CD) were recorded using a DSM 16 CD spectroplarimeter with a modified Cary 118 monochromator (Olis Inc., Bogart, GA). The monochromator, lamp-housing and sample-compartment were thoroughly purged with nitrogen gas from a liquid-N_2 tank prior to and during data acquisition. All spectra were recorded in the Soret band region and blank subtracted.

2.2.1.2. Time-resolved experiments. Fluorescence decay lifetimes were measured using a time-correlated single photon counting (TCSPC) instrument (Fluorocube, Horiba Scientific, Edison, NJ) with a 294 nm pulsed LED (NanoLED-293, Horiba JobinYvon, Edison NJ) source of ~1 ns pulse width and 1 MHz repetition rate. Decays were recorded at the emission maximum of HSA with a 12 nm bandwidth.

2.2.1.3. Fluorescence quenching. Binding between porphyrins and proteins can be investigated by recording the quenching of the intrinsic fluorescence of the protein as a function of the concentration of the porphyrins (i.e., quencher). From the analysis of the quenching, one can extrapolate whether the mechanism is collisional or static and in the second case establish the binding constant [29]. Fluorescence quenching of HSA by TSPP was measured by recording the emission of the protein upon addition of increasing aliquots of an aqueous stock of the porphyrin to the solution containing HSA (~ 4 μM). The concentration of TSPP is determined from the value of its absorption maximum using the molar extinction coefficient reported above. Emission spectra of HSA were recorded between 300 and 450 nm with $\lambda_{ex}=295$ nm (where one can assume exclusive excitation of the lone Trp214 residue [30]). The selected protein concentration ensures an $OD_{295} < 0.1$.

In order to distinguish between the contributions of dynamic and static quenching, we examined the effect of temperature by performing quenching experiments at 22 °C as well as 30 °C and 40 °C using aliquots of the same porphyrin and protein stocks. Temperature was changed using a Peltier temperature control element (SPG 1A, Thermo Fisher Scientific, Waltham, MA) in absorption, and a water circulator (Polystat, Cole Parmer, Vernon Hills, Il) in fluorescence. The setting in both devices were kept as to ensure the same equilibration temperature in the two instruments.

Throughout the sample preparation and measurement, the lights in the laboratory were dimmed in order to ensure minimal ambient light exposure of the sample (measured at ~ 50 μW/cm^2 in the areas where samples were handled) as to avoid photochemical effects on the porphyrin and the protein [24].

2.2.1.4. Fluorescence quenching analysis. Fluorescence quenching constants were calculated using the Stern-Volmer (S-V) Eq. (1) [31].

$$\frac{F_0}{F}=1 + K_Q[Q] \quad (1)$$

where F is the total fluorescence intensity of HSA and F_0 indicates the fluorescence before addition of the quencher, K_Q is the quenching constant, and *[Q]* is the concentration of the porphyrin determined spectrophotometrically as described above. K_Q is obtained from the slope of $\frac{F_0}{F}$ vs. *[Q]*. Additional information of the quenching experiments are provided in the Supplementary Material.

2.2.1.5. Porphyrin fluorescence. In addition to probing the fluorescence quenching of the protein, interactions of porphyrins with proteins can be investigated by recording the changes in the