

Stability of spectrofluorimetric spectra of hematoporphyrin–serum albumin complexes: *in vitro* study

Leszek Sułkowski¹, Andrzej Matyja², Czesław Osuch², Maciej Matyja²

¹Department of General Surgery, Regional Specialist Hospital, Częstochowa, Poland

²Department of General Surgery, Jagiellonian University Medical College, Krakow, Poland

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Corresponding author:

Leszek Sułkowski

Department of

General Surgery

Regional Specialist Hospital

Bialska 104/118

42-218, Częstochowa, Poland

Phone: +48 792244177

E-mail: leszeksulkowski@icloud.com

Abstract

Introduction: Hematoporphyrin is a photosensitizer used in photodynamic therapy of various malignant diseases. It is carried to the cancer tissue by serum albumins. Spectrofluorimetric spectra of hematoporphyrin–serum albumin complexes were examined *in vitro*.

Material and methods: The chemicals were: hematoporphyrin, human serum albumin and bovine serum albumin. The spectra were recorded on a Kontron SFM-25 Instrument AG at two excitation wavelengths: $\lambda_{ex} = 280$ nm and $\lambda_{ex} = 295$ nm. The spectra of hematoporphyrin 1.5×10^{-5} M as well as spectra of complexes of hematoporphyrin–human serum albumin (1.5×10^{-5} M Hp – 1.25×10^{-6} M HSA) and hematoporphyrin–bovine serum albumin (1.5×10^{-5} M Hp – 3.5×10^{-7} M BSA) were recorded repetitively for 8 days and compared to the initial spectrum.

Results: Formation of a complex with human serum albumin extends the stability of the hematoporphyrin spectrum. This extension is greater at excitation $\lambda_{ex} = 295$ nm. Different stability of complexes with bovine and human serum albumins most likely does not result from an actual lower stability of bovine serum albumin complexes, but from the fact that dissimilarity in the structure of both albumins enables additional spectroscopic observations within subdomain IB in the bovine serum albumin molecule.

Conclusions: Spectrofluorimetric spectra are stable longer when hematoporphyrin forms a complex with human serum albumin. The present data may be important for understanding the mechanism of hematoporphyrin transportation to the target cancer tissue and effectiveness of photodynamic therapy.

Key words: photodynamic therapy, photosensitizer, spectrofluorimetry, hematoporphyrin, human serum albumin, bovine serum albumin, binding protein.

Introduction

Photodynamic therapy is a minimally invasive treatment modality for a variety of malignant diseases and precancerous lesions of skin, breast, esophagus and colon, urinary bladder, head and neck [1–5]. Exogenously administered photosensitizer accumulates in tumors and selectively sensitizes cancer tissue to the laser light. This phenomenon of photosensitization leads to formation of cytotoxic compounds and damage of cancer cells [4–7].

Porphyrins are one of the best studied groups of photosensitizers [2]. Porphyrin molecules are composed of pyrrole rings bridged by hybridized carbon atoms [8]. Porphyrins have been applied in clinical practice as photodetectors and photosensitizers in photodynamic diagnosis and therapy [7]. Hematoporphyrin (Hp) is an intravenously administered, organic porphyrin photosensitizer, which inhibits proliferation of endothelial cells and induces apoptosis [2, 3, 9].

Photosensitizers are carried to the cancer tissue by serum albumins, which bind reversibly and carry various endogenous and exogenous compounds in the circulatory system [2, 8–10]. Pharmacokinetics and side effects of photosensitizers depend on their ability to bind serum albumin and reach the target cancer cells [8, 9, 11]. Formation of a complex between photosensitizer and serum albumin improves solubility and transportation, reduces toxicity and protects the photosensitizer against oxidation [10].

Human serum albumin (HSA) contains 585 amino acid residues and forms an equilateral triangle shape consisting of three domains: I, II and III. Each of them forms subdomains A and B [9, 12–16]. Among serum albumins of other species, the structure of bovine serum albumin (BSA) is the most similar to HSA [9, 12]. The amino acid sequences of HSA and BSA are 76% homologous [9, 17]. From a spectroscopic point of view, tryptophanyl residues, which are fluorophores, are important. The HSA molecule contains one tryptophanyl residue (Trp214), while BSA contains two (Trp135 and Trp214) [9, 10, 12]. Trp135 is located in subdomain IB, while Trp214 is located in subdomain IIA [11]. This dissimilarity between HSA and BSA enables additional spectroscopic observations [17, 18].

Binding to serum albumin affects Hp distribution to the cancer tissue, the efficiency of the photodynamic response and finally the success of photodynamic therapy [2, 7, 8]. The aim of this study was to examine *in vitro* the stability of spectrofluorimetric spectra of Hp-HSA and Hp-BSA complexes.

Material and methods

Chemicals

The chemicals were:

- hematoporphyrin (Hp), molecular weight 598.71 Da, obtained from Sigma-Aldrich Inc. St. Louis, USA,
- human serum albumin (HSA), fraction V, molecular weight 67 000 Da, crystallized and lyophilized, obtained from ICN Biomedical Inc. Aurora, OH, USA,
- bovine serum albumin (BSA), molecular weight 66 500 Da, obtained from Biomed Lublin, Poland.

The highest Hp solubility has been experimentally observed in sodium phosphate buffer 0.05 M, pH 7.40. In order to study the stability of complexes with Hp the solutions in this buffer of both serum albumins were prepared.

Apparatus

The emission fluorescence spectra were recorded on a Kontron SFM-25 Instrument AG (Kontron AG, Zurich, Switzerland) with $1 \times 1 \times 4$ cm quartz cells. The spectra were recorded 30 min after sample preparation, at 25°C. Correcting error of the apparatus for wavelength was $\lambda = \pm 1$ nm, while for the relative fluorescence $RF = \pm 0.01$. The concentration was adjusted to produce an absorbance $A \leq 0.05$ and the fluorescence spectra were not corrected for the inner filter effect [19]. To excite fluorophores two excitation wavelengths, $\lambda_{ex} = 280$ nm and $\lambda_{ex} = 295$ nm, were used. The scan ranges were 280–400 nm and 295–400 nm, respectively, for these excitation wavelengths.

Emission fluorescence spectra

The emission fluorescence spectra of Hp, HSA and BSA in sodium phosphate buffer 0.05 M at concentrations 1.5×10^{-5} M, 1.25×10^{-6} M and 3.5×10^{-7} M, respectively, were recorded repetitively for 8 days for both $\lambda_{ex} = 280$ nm and 295 nm. The emission fluorescence spectra of solutions 1.5×10^{-5} M Hp – 1.25×10^{-6} M HSA and 1.5×10^{-5} M Hp – 3.5×10^{-7} M BSA were recorded repetitively to study the stability of Hp-HSA and Hp-BSA complexes.

Results

The peak fluorescence on the day of sample preparation was computed as 100%. During the following 8 days, the consecutive peaks were compared to the initial value. The change exceeding 5% of the initial one was found to be significant.

The peak fluorescence of Hp excited at $\lambda_{ex} = 280$ nm and $\lambda_{ex} = 295$ nm changed significantly on the second and fourth day after sample preparation, respectively (Figure 1; lines 1 and 2).

The peak fluorescence of the Hp-HSA complex changed significantly on the third and seventh day for $\lambda_{ex} = 280$ nm and 295 nm, respectively (Figure 1; lines 3 and 4). The peak fluorescence of Hp-BSA complex excited at $\lambda_{ex} = 280$ nm and 295 nm changed significantly on the second and third day following sample preparation, respectively (Figure 1; lines 5 and 6).

Discussion

Emission spectrum is a sensitive detector of changes within fluorophores, which tyrosines

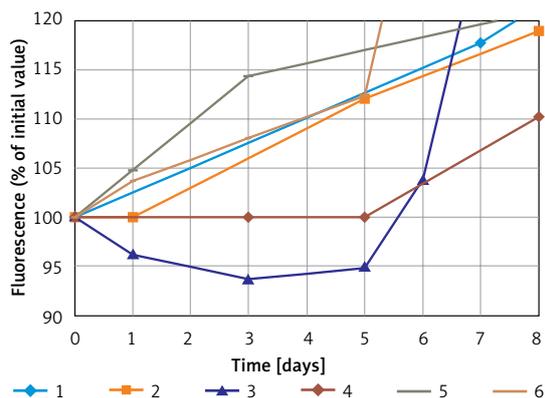


Figure 1. Time related changes of emission fluorescence spectra of: Hp excited at $\lambda_{\text{ex}} = 280$ nm (line 1) and 295 nm (line 2); Hp-HSA complex excited at $\lambda_{\text{ex}} = 280$ nm (line 3) and 295 nm (line 4); Hp-BSA complex excited at $\lambda_{\text{ex}} = 280$ nm (line 5) and 295 nm (line 6)

(Tyr) and tryptophans (Trp) are [8, 13, 14, 17]. At $\lambda_{\text{ex}} = 280$ nm both Tyr and Trp are excited, while at $\lambda_{\text{ex}} = 295$ nm only Trp is [8, 12, 13]. It was also observed that fluorescence quenching of both serum albumins is positively correlated with Hp concentration and may be explained by the energy transfer from HSA and BSA fluorophores to Hp chromophores [2, 7, 8]. The transfer of energy may appear when the distance between Hp and serum albumin does not exceed 10 nm [9, 11, 20, 21]. Two drug binding sites described by Sudlow are: hydrophobic, very flexible site I containing Trp214 (in both HSA and BSA) and smaller, less flexible site II containing Trp135 (present only in BSA) [13, 15, 16, 18]. Spectroscopic information for HSA can only be obtained from subdomain IIA, where Trp214 is located [9, 11, 12]. BSA, which structurally is the most similar to HSA, contains in the amino acid sequence two Trps (Trp135 and Trp214), instead of only one, Trp214, in HSA [2, 12, 13]. This structural dissimilarity between the two albumins enables spectroscopic information to be obtained from both subdomains IIA and IB when BSA is studied [9, 12, 13, 17].

Previous *in vitro* spectrometric studies have shown that porphyrins form complexes with serum albumins [2, 7, 8]. Three structurally homologous domains could be found in the tertiary structure of serum albumins. Each domain is formed by two subdomains: A and B [12]. Hp has probably two classes of binding sites located in subdomains IB and IIA [2]. The Hp spectra remain stable for at least 1 or 2 days following sample preparation at $\lambda_{\text{ex}} = 280$ nm and 295 nm, respectively (Figure 1; lines 1 and 2). However, it is known that even such stability may be sufficient, because in clinical settings the photosensitizer is infused a few hours before exposure to the light [20].

In this experiment it was observed that when the Hp-HSA complex is formed, stability of spectra extends to 2 and 6 days following sample preparation at $\lambda_{\text{ex}} = 280$ nm and 295 nm, respectively (Figure 1; lines 3 and 4). In previous studies it was suggested that Hp forms complexes with both HSA and BSA [2, 8]. The phenomenon of complex formation is the most likely explanation of extended stability of spectrofluorimetric spectra.

Shorter stability of spectra was observed in the case of the Hp-BSA complex (Figure 1; lines 5 and 6). However, most likely this phenomenon results not from an actual lower stability of Hp-BSA complexes, but from the fact that BSA contains additional Trp135 in subdomain IB, which can also be observed spectroscopically [9, 12, 13, 17]. The stability of both Hp-BSA and Hp-HSA complexes is sufficient to allow delivery of the photosensitizer to the target tissue [20].

In conclusion, the fluorescence spectrum of Hp is stable longer when it forms a complex with HSA. The observed phenomenon may be important for understanding mechanisms of Hp transportation to the target cancer tissue and the effectiveness of photodynamic therapy.

Conflict of interest

The authors declare no conflict of interest.

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