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The Effect of Serum Albumin on Binding of Protoporphyrin IX to Phospholipid Membrane

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The protoporphyrin (PPIX) incorporated liposomes were prepared and the change of PPIX UV-Vis spectra in this system were analyzed. The effect of serum albumin (BSA) on the lipo-PPIX system has been presented. Different changes in absorbance of peaks I (at λ_{max} 279 nm) and II-V, observed in the lipo-PPIX spectrum in the presence of BSA, suggest that PPIX associated with liposome exists in two different microenvironments, i.e., hydrophobic and polar. The latter is located near the membrane surface close to the polar phospholipid head. The release of the PPIX from these sites is not observed in the presence of BSA.

Time dependence of the spectral properties of solution PPIX in water has been presented. It was found that BSA forms a complex with PPIX at PPIX/BSA molar ratio 0.2/1.

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INTRODUCTION

Porphyrins and related compounds have already been applied in medicine for a number of years as photodetectors in photodynamic diagnosis of cancerous tissues [1–3] and as photosensitizers in the photodynamic therapy of cancer [2,4,5]. Photomodification of biological membranes is one of the critical processes leading to cellular damage upon photodynamic treatment of living cells [6]. Association of the dyes (e.g., porphyrins) with membrane surface is expected to enhance the effectiveness of the photodynamic action on the membrane [7].

Porphyrins are usually introduced into blood as solutions. The binding constant K_a for PPIX-serum albumin complex is equal to $6 \times 10^5\,M^{-1}$ [8]. Hence the interaction of serum albumin with PPIX may alter the efficiency of its photodynamic action.

Our aim was to present the changes of the spectral properties of PPIX incorporated into phospholipid membranes and the effect of serum albumin on this system.

MATERIALS AND METHODS

Materials

Soy-bean lecithin was obtained from Lucas Mayer GmbH, bovine serum albumin (BSA) crystallized and lyophylized, fraction V from BIOMED WSIS (Lublin) and protoporphyrin IX (PPIX) (3,7,12,15-tetramethyl-8, 13-divinyl-2, 18-porphyrine-dipropionic acid) from Aldrich GmbH.

Methods

For preparation of liposomes a soya-bean lecithin (EPICURON 14V-Lucas Mayer) and cholesterol (molar ratio 2:1 I 2:1.5) were applied. The liposomes were prepared by the modified reverse-phase evaporation method [9]. Then extrusion technique with a small-volume of extrusion apparatus Lipo-Fast-Basic prod. Avestin Inc. was used [10]. Protoporhyrin (PPIX) and bovine serum albumin (BSA) were added to the reagent mixture during the preparation. For each experiment with lipo-BSA-PPIX five control preparations were carried out i.e. liposome, liposome-BSA, liposome-PPIX and two preparations where instead of soy-bean lecithin the respective BSA and PPIX quantities were added.

UV-Vis Absorption Spectra

The UV-Vis spectra were recorded at room temperature with JASCO V-530 spectrophotometer using cells 1 cm × 1 cm × 4 cm. The accuracy of the absorbance and wavelength is 0.02 and 0.5 nm, respectively.

With exception of the time-dependence experiment, all samples were freshly prepared.

RESULTS AND DISCUSSION

Time Dependence

Time dependent UV-Vis spectra of PPIX are shown in Figure 1. The absorption peaks are denoted as follows: I·281–285 nm, II·371–365 nm, III·472–470 nm, IV·539 nm, V·591 nm, and VI·642 nm. The change of absorbance and λ_{max} for each PPIX absorption peak in the time range 40 min÷24 h is shown in Figures 2A and B, respectively. Only a slight hyperchromic effect is observed after 24 h incubation for peaks IV, V, and VI, i.e., by 0.09, 0.08, and 0.06, respectively (Fig. 2A). The significant increase of absorbance by 0.16 is seen after 24 h incubation for signal III. The most distinct decrease of absorbance by 0.75 is observed for the PPIX UV absorption peak II (Fig. 2A). It is accompanied by a blue shift of 6 nm (insert in Fig. 2B). The λ_{max} of the remaining PPIX signals does not change with time (Fig. 2B). However, for all signals of PPIX absorbance and λ_{max} are constant within 110 min incubation. Therefore, in the experiments that followed this time limit was not excideed.

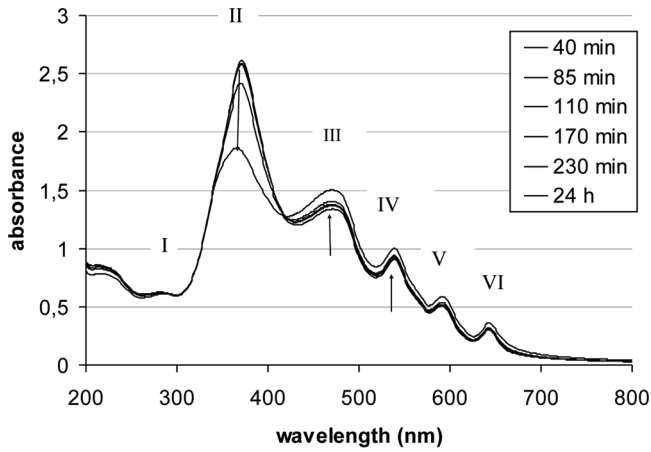

FIGURE 1 UV-Vis spectra of PPIX recorded after 40 min÷24 h incubation.

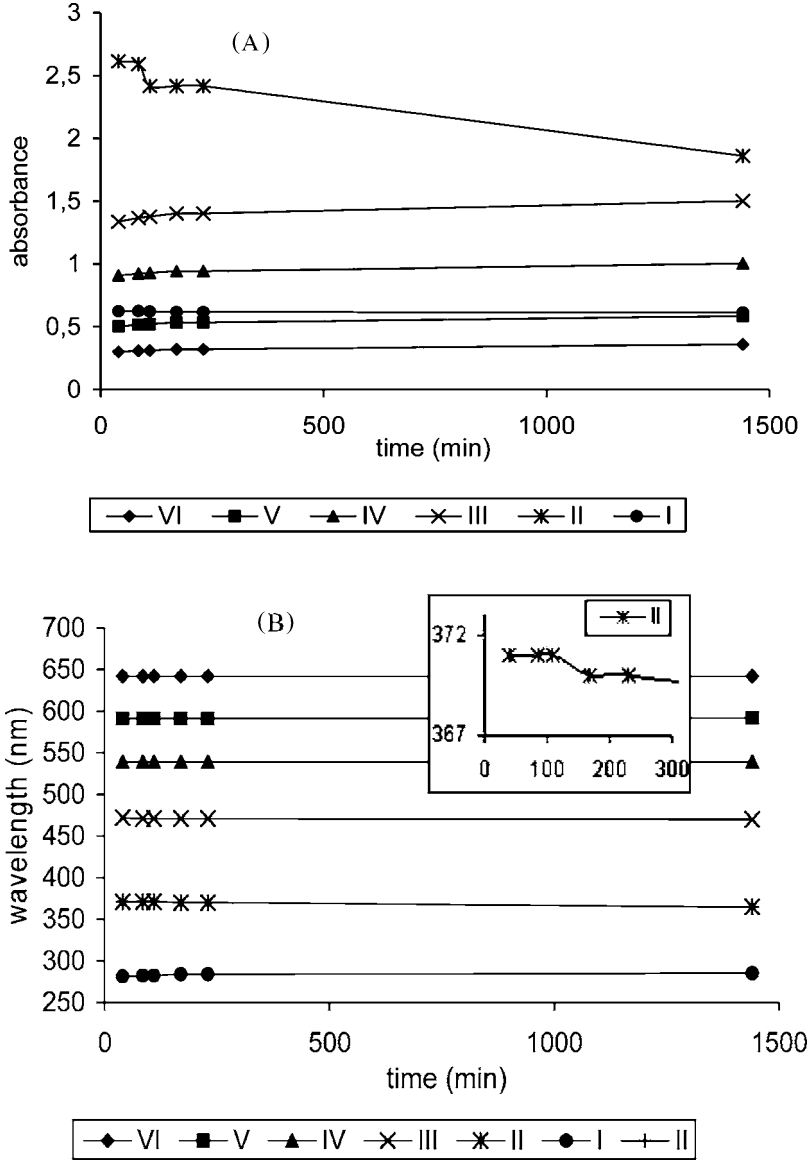

FIGURE 2 Time dependence of absorbance (A) and wavelength (B) for each PPIX peak. In the insert the time dependence for peak II is shown. The error bars (instrumental accuracy) are smaller than the symbols.

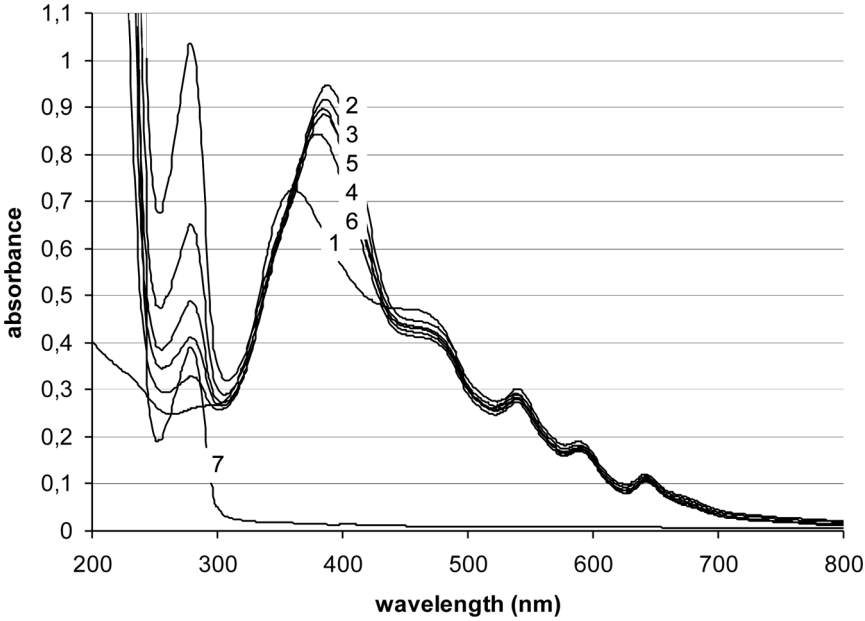

FIGURE 3 UV-Vis spectra of PPIX [2×10^{-5} M] (1), BSA [1×10^{-5} M] (7) and PPIX/BSA systems at following molar ratios: (2) 1:1, (3) 0.5:1, (4) 0.3:1, (5) 0.2:1, and (6) 0.1:1.

BSA-PPIX Interactions

In order to analyze the binding of PPIX to the phospholipid membrane in the presence of serum albumin the changes in the microenvironment of PPIX chromophores in the presence of BSA were first examined.

The PPIX-BSA system of three PPIX/BSA molar ratios 10/2÷80, 20/2÷20, and 30/2÷20 has been analyzed with the use of UV-VIS spectroscopy. In Figure 3 the UV-VIS spectra of PPIX-BSA complexes of molar ratio BSA/PPIX 0.1÷1/1 (spectra 2–6) are shown on the basis of spectra of BSA [1×10^{-5} M] (spectrum 7) and PPIX [2×10^{-5} M] (spectrum 1).

The significant changes of A and λ_{max} are observed for PPIX absorption peaks I and II. Changes of absorbance of the signal I (~ 280 nm) could be connected with the increasing protein concentration. However, the increase of absorbance of signal II at constant PPIX concentration accompanying by the batochromic effect by 27 nm shown in Figure 3 for PPIX peak II pointed to the complex formation.

Only a slight hypochromic effect for PPIX is observed for absorption peak III.

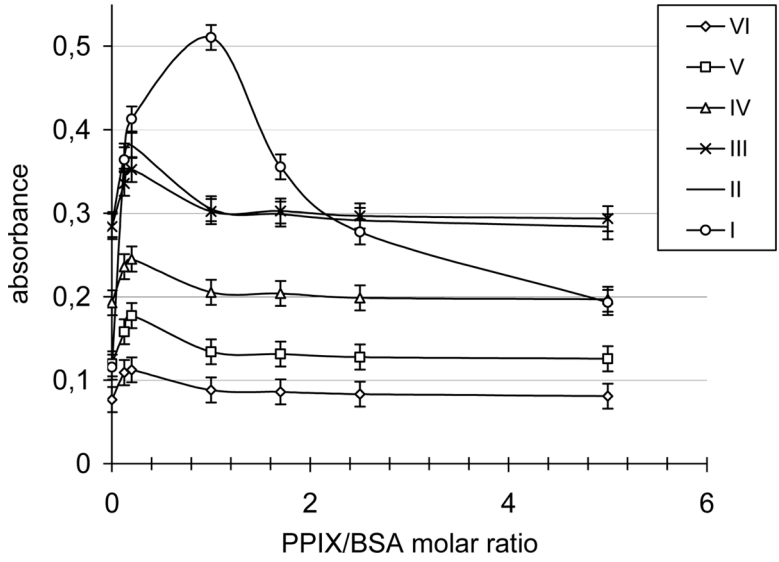

FIGURE 4 The dependence of absorbance of each PPIX peak on the PPIX/BSA molar ratio.

If the interaction with BSA exists it induces changes in the microenvironment. The quenching effect of PPIX on BSA fluorescence, described previously [8], confirms this suggestion. Moreover, the chromophores coupled to PPIX absorb light and, due to energy excess, may induce photodynamic effects by transfer of the excitation energy. Detailed changes in absorbance of each UV-Vis absorption peak of PPIX due to the formation of the complex with BSA can be seen in Figure 4.

The changes of peak I observed in Figure 4 cannot be interpreted since this peak appears in both PPIX and BSA spectra. But for all the remaining peaks of PPIX the rise of absorbance at PPIX/BSA molar ratio 0.2 is noted (Fig. 4). This regularity could be the result of the formation of the complex at the mentioned molar ratio.

PPIX Binding to Lipid Bilayer

In Figure 5 the spectra of PPIX free (6), of PPIX bound to a phospholipid membrane (5), of PPIX bound to the liposome entrapped BSA (4), of BSA- liposome system (3) and of the free BSA (2), obtained after 15 min incubation, are shown. The arrows in Figure 5 show that the

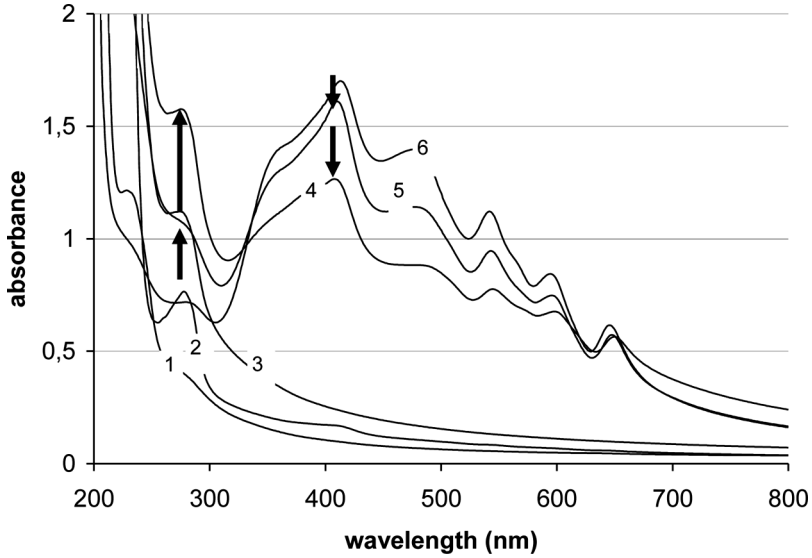

FIGURE 5 UV-Vis spectra of PPIX: (6) – free, (5) – bound to the phospholipid membrane, (4) – bound to BSA encapsulated membrane; (1), (2), and (3), – UV-VIS spectra of liposome, BSA and liposome-BSA system, respectively. Arrows show changes in absorbance of PPIX in the following systems: PPIX → lipo-PPIX → lipo-BSA-PPIX.

absorbance of signal I increases and signals II–V decrease for the spectra of systems in following sequence:

PPIX → lipo-PPIX → lipo-BSA-PPIX

The absorbance is lower for peaks II–V when PPIX is bound to the membrane (spectrum 5 in Fig. 5) than for the free PPIX spectrum (spectrum 6). On the contrary, absorbance of peak I at λ_{max} at 280 nm rises in the same systems. This phenomenon probably points to the immobilization of PPIX in two different microenvironments in the membrane. In the presence of BSA absorbance of four peaks (II-V) of the membrane bound PPIX (spectrum 4 in Fig. 5) decreases in comparison to the lipo-PPIX system (spectrum 5 in Fig. 5) whereas absorbance of peak I (280 nm) increases (spectrum 4 in Fig. 5). This effect of BSA on the PPIX incorporated liposome spectrum can be explained by the existence of PPIX in two sites in the phospholipid membrane which differ in their accessibility to the protein.

The fact that serum albumin causes the release of adenosine derivatives [10,11] and metronidazole [12] encapsulated into liposomes has been previously described.

To assess if serum albumin acts in the same way on the PPIX incorporated membrane the spectra of lipo-PPIX (spectrum 5 in Fig. 5) and lipo-PPIX-BSA (spectrum 4 in Fig. 5) are compared. The release of PPIX from membrane should result in the increase of PPIX absorbance. Since this effect is not observed in the presence of BSA (spectrum 4 in Fig. 5) in comparison to the spectrum of lipo-PPIX (spectrum 5 in Fig. 5) system, one can conclude that the release of PPIX from the membrane is not easier in the presence of BSA. This is probably due to additional BSA-PPIX interactions.

CONCLUSIONS

Time dependence of the spectral properties of PPIX solution in water is presented. Although the essential changes in the UV spectra appear during the first 4 h of incubation for all signals of PPIX, absorbance and λ_{max} are constant within 110 min. of incubation. Therefore, in our experiments this time limit was not exceeded.

It is shown that BSA forms a complex with PPIX at molar ratio PPIX/BSA 0.2/1.

Various changes in absorbance for peaks I and II-V i.e., increase of absorbance of signal I and decrease of signals II-V for the spectra of systems in the following sequence:

PPIX → lipo-PPIX → lipo-BSA-PPIX may suggest that PPIX associated with liposome exists in two different microenvironments i.e., hydrophobic and polar. The latter located near the membrane surface close to the polar phospholipid head. The release of the PPIX from these sites is not observed in the presence of BSA.

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