Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 3189–3192
Bioorganic & Medicinal Chemistry Letters

Investigation of human serum albumin (HSA) binding specificity of certain photosensitizers related to pyropheophorbide-a and bacteriopurpurinimide by circular dichroism spectroscopy and its correlation with in vivo photosensitizing efficacy

Yihui Chen,[a] Razvan Miclea,[b] Thamarapu Srikrishnan,[c] Sathyamangalam Balasubramanian,[b] Thomas J. Dougherty[a] and Ravindra K. Pandey[a,d,*]

[a]*Chemistry Division, Photodynamic Therapy Center, Roswell Park Cancer Institute, Buffalo, NY 14263, USA*
[b]*Pharmaceutical Sciences Program, SUNY, Buffalo, NY 1422, USA*
[c]*Department of Cancer Biology, Roswell Park Cancer Institute, Buffalo, NY 14263, USA*
[d]*Nuclear Medicine and Radiology, Roswell Park Cancer Institute, Buffalo, NY 14263, USA*

Received 6 January 2005; revised 4 May 2005; accepted 4 May 2005

Abstract—A series of pyropheophorbide-a and bacteriopurpurinimides were investigated to understand the correlation between HSA (site II) binding affinity and in vivo photosensitizing activity. In our study, photosensitizers that bound to site II of HSA produced a significant difference in the circular dichroism spectra of the corresponding complexes, especially at Soret band region of the photosensitizers. Our results suggest that CD spectroscopy of the photosensitizer–HSA complexes could be a valuable tool in screening new photosensitizers before evaluating them for in vivo efficacy.

1. Introduction

Circular dichroism (CD) is a powerful measurement that can detect the chiral structure.[1] CD is observed when optically active matter absorbs left- and right-hand circular polarized light slightly differently. It is measured with a CD spectropolarimeter. The CD is a function of wavelength. For a compound to produce circular dichroism, it should have asymmetry and an electronic absorption. Due to the extended conjugated π-electron systems, chlorins and bacteriochlorins have obvious electronic absorptions within a wide range (300–900 nm). Also because one (chlorin) or two (bacteriochlorin) double bonds are reduced to the single bonds, chlorins and bacteriochlorins are intrinsically asymmetric. Therefore, CD has the potential to be a powerful tool for investigating these novel structures.

The investigation of drug–protein interactions and the determination of binding parameters are of fundamental importance in establishing the mechanism of action of a drug as well as in accessing the extent of its activity at the target site. In the case of plasma proteins, which act as drug carriers in the body, binding interactions have a profound effect on overall drug activity. Among all the proteins, human serum albumin (HSA) is the most abundant plasma protein.[2] Many compounds, especially amphiphilic drugs and some endogenous substances, bind reversibly and with high affinity to HSA.[3] The formation of this complex decreases the concentration of unbound molecules in the plasma thereby effecting the drug's distribution, pharmacokinetics, toxicity, and ultimately, its rate of excretion. Reversible binding to HSA extends the lifetime of a drug in plasma but it also decreases the concentration of the free drug available for physiological action. Thus, from the pharmacokinetic point of view, binding to HSA is beneficial only as long as one extends the length of time at which the free drug remains above its therapeutically effective concentration. Such a benefit may especially be important for drugs with low solubility in plasma for which HSA will act as a slow-delivery reservoir. On the other hand, if the binding is too tight and release is too slow, it will seriously restrict the action of the drug. It is known that

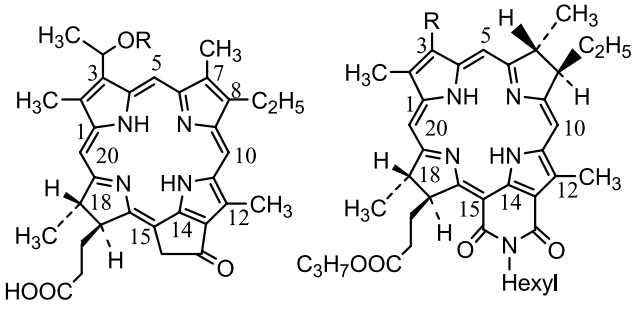
* Corresponding author. Tel.: +1 7168453203; fax: +1 7168458920; e-mail: ravindra.Pandey@RoswellPark.org

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.05.019

human serum albumin binding affinity of various photosensitizers plays an important role in their biodistribution within the tumor stroma.[4,5] The development of an in vitro method, which would allow the rapid quantitation of the binding affinities of drugs toward HSA, would be particularly useful for the identification of potential in vivo pharmacokinetic problems.[3]

Circular dichroism has been widely used to monitor the binding of small organic ligands to serum proteins, by virtue of the contribution that can be generated upon binding.[6] This induced CD can provide information on the binding affinity, the conformation of the bound ligand, and the possible protein conformational changes. In addition, this technique can be used to detect the interaction between ligands in their binding to the protein. The induced CD can be observed, either for achiral or chiral drugs, at the wavelengths where the electronic transitions of the drug chromophores occur. In the case of an achiral drug, the contribution to the CD may arise from the stabilization of one of the chiral conformers in which the drug is in equilibrium, or in general from an asymmetric perturbation of the chromophores by the electrostatic field of the nearby amino acid residues. In the case of a chiral drug, the extrinsic Cotton effect will be added to the intrinsic one and the interpretation of the resulting induced ellipticity becomes more difficult. In fact, the extrinsic Cotton effect may arise from either the induction of a different conformation in the bound drug or a different polarity of the protein binding site that results in different solvation of the drug chromophores when bound to the protein and, therefore, CD bands observed.

The present work describes the CD spectral changes of certain chlorins and bacteriochlorins after binding to HSA and a correlation between the HSA (site II) binding values and PDT specificities and photodynamic therapeutic (PDT) efficacies. The CD spectra were run on a JASCO-600 spectrometer.



1. R=-$(CH_2)_5CH_3$, n-Hexyl Ether Analog of Pyropheophorbide-a (HPPH)
2. R=-$(CH_2)_6CH_3$, n-Heptyl Ether Analog of Pyropheophorbide-a (Heptyl)
3. R=-$(CH_2)_2CH{=}CHC_2H_5$, *cis*-3-Hexenyl Ether Analog of Pyropheophorbide-a (Hexenyl)
4. R = -$COCH_3$
5. R = -$CH(OH)CH_3$
6. R = -$CH(OCH_3)CH_3$
7. R = -$CH(OC_3H_7)CH_3$
8. R = -$CH(OC_7H_{15})CH_3$
9. R = -$CH(OC_{10}H_{21})CH_3$

Scheme 1. The structure of compounds **1**–**9**.

Pyropheophorbide-a analogs **1**–**3**[7] and bacteriopurpurinimides **4**–**9**[8] were prepared by following the methodologies developed in our laboratory (Scheme 1).

2. Results and discussions

Several binding sites in HSA have been identified.[9–11] In the studies of small organic molecules, two major binding sites on HSA were found. The site binding with 5-dimethylaminonaphthalene-1-sulfonamide (DNSA) was named "site I". This site is also the binding site for warfarin and azapropazone. The site binding with dansyl-L-proline (DP) was named "site II". Site II was also diazepam or tryptophan binding site. Binding sites I and II are located at subdomains IIA and IIIA, respectively. Binding of ligands to these sites is often selective.[3]

In our initial study, three chlorophyll-a derived photosensitizers **1**, **2**, and **3** were selected for investigating their relationship between binding to site I and site II of HSA and in vivo PDT efficacy. From the results summarized in Table 1, it can be seen that compound **1** (HPPH), bound to both site I and site II of HSA; compound **2**, that is, the heptyl analog, only bound to site II; while compound **3**, that is, the hexenyl analog, did not bind to site II; instead, it binds to site I only.

It is interesting to observe that both photosensitizers **1** and **2**, which bind to site II of HSA also produced good in vivo PDT efficacy (Table 1). In contrast, compound **3**, that does not bind to site II of HSA, showed no efficacy.

Interesting results were also obtained on investigating the circular dichroism spectra of compounds **1**, **2**, and **3** (Fig. 1). It was noted that from ~310 nm to a shorter wavelength, pure HSA exhibits a very strong negative circular dichroism absorption, so the CD spectrum of the complex in that region is predominated by HSA. Contrarily, from ~310 nm to longer wavelength, the CD spectrum of the complex is dominated by the photosensitizer part. Analyzing the CD spectrum of the complex formed by **3** and HSA (Fig. 1C), it is found that its spectrum on the region from ~310 nm to longer wavelength is exactly similar to the spectrum of pure compound **3**, while its spectrum on the region from ~310 to shorter wavelength resembles the spectrum of pure HSA. These results suggest that, the two components,

Table 1. The HSA binding data and in vivo PDT efficacies of three chlorophyll-a analogs; mice were transplanted with tumors

Drug	HSA binding affinity		Tumor response (%) 10 mice (C3H mice with RIF tumors)/group[a]			
	Site I	Site II	Day 7	Day 14	Day 30	Day 90
1	Yes	Yes	100	100	50	50
2	No	Yes	100	100	80	50
3	Yes	No	0	0	0	0

The tumors were injected a drug dose of 0.47 μmol/kg. The tumors were exposed with laser light (135 J/cm^2) at 665 nm for 30 min, 24 h post-injection of the drug.
[a] 50% tumor response means 5/10 mice were tumor free.

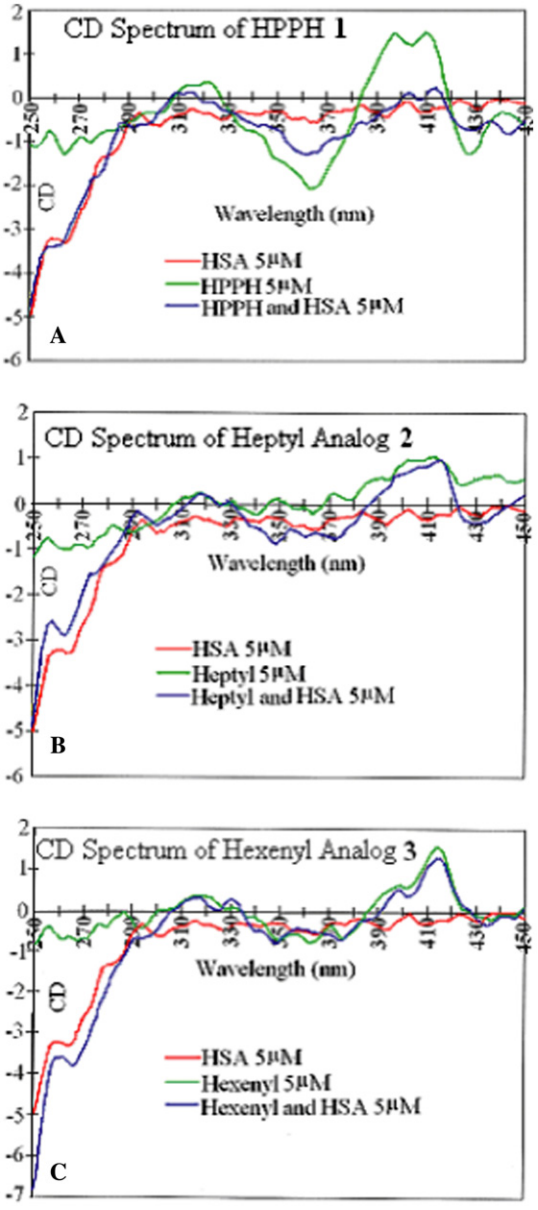


Figure 1. Circular dichroism spectra of HSA, compounds **1**, **2**, and **3** and the corresponding complexes (HSA + photosensitizers).

HSA and compound **3**, have no interaction with each other, which could be due to the fact that the compound **3** does not show any affinity to site II of HSA (see Table 1 and Fig. 1C).

For the site II (HSA) binding photosensitizers **1** and **2**, definite CD spectral changes on the complexes formed by photosensitizers and HSA (Figs. 1A and B) were observed. These changes were mainly noticed at approximately 400 nm region (also called 'Soret' band)[12], indicating a possible binding of these photosensitizers with aromatic residues in HSA.[11,12] For compound **1**, the most conspicuous changes are at 360 and 400 nm, while for compound **2**, the most notable changes are at 425 nm (Fig. 2). The HSA binding constant values are: for compound **1**: $1.1 \times 10^7\ M^{-1}$ (site I), $1.0 \times 10^8\ M^{-1}$ (site II); for compound **2**: $2.0 \times 10^7\ M^{-1}$

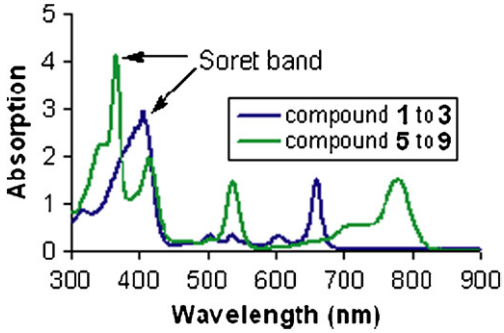


Figure 2. Electronic absorption spectra of photosensitizers related to pyropheophorbides **1**–**3** and bacteriopurpurinimides **5**–**9** (in MeOH).

(site II, no site I binding) and for compound **3**: $0.98 \times 10^7\ M^{-1}$ (site I, no site II binding was observed).

From the results discussed above in which **1**, **2**, and **3** were used as substrates, with our previous investigations on various Photofrin® components[13] and benzobacteriochlorins,[14] it can be seen that the preferentially binding to site II (not site I) of HSA plays an important role in the in vivo PDT efficacy. To further explore the correlation between HSA (site II) binding and PDT efficacy in other photosensitizers, a series of bacteriochorin derivatives **4**–**9** were also investigated, and the CD spectroscopy of the corresponding HSA complexes was analyzed.

From the results summarized in Table 2, it can be seen that compounds **5**–**9** competitively displaced DP (dansyl-L-proline), the site II probe of HSA, with the binding constant values ranging from 0.964×10^7 to $2.09 \times 10^7\ M^{-1}$, while compound **4** did not bind to site II of HSA. Among the bacteriochlorins investigated, except for compound **5**, a correlation between the PDT efficacy and HSA binding was observed. This could be due to a significant difference in pharmacokinetic characteristics of the photosensitizer.

The dichroism spectra of **5**–**9** were similar to each other. The difference in the length of carbon chain of the alkyloxy groups present at position-3 did not have any effect in their CD spectra. The CD spectral changes at the Sor-

Table 2. The HSA binding results and in vivo PDT efficacies of a series of bacteriochlorin derivatives **4**–**9**

Drug	HSA site II binding constant K_b[13] ($\times 10^7\ M^{-1}$)	Tumor response[8] (%) 6 mice (C3H mice with RIF tumors)/group[a]			
		Day 7	Day 14	Day 30	Day 90
4	No binding	Not effective			
5	0.964	Not effective			
6	1.47	100	83	0	0
7	2.09	100	100	0	0
8	1.11	100	100	83	83
9	1.26	100	100	33	33

[a] Mice were injected the drug dose of 0.4 μmol/kg and the tumors were treated with a laser light (796 nm, 135 J/cm^2, 75 mW) for 30 min at 24 h post-injection. 83% tumor response means 5/6 mice were tumor free.

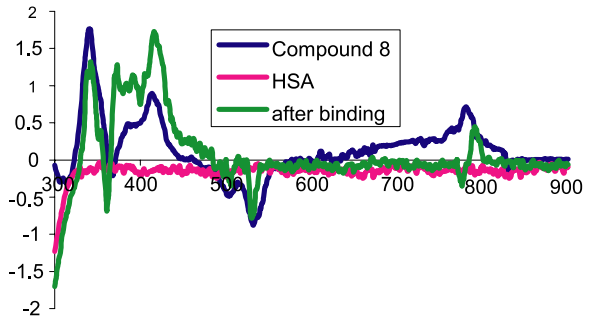


Figure 3. The CD spectra of bacteriochorin **8** before and after binding to HSA. For a general procedure see Ref. 15.

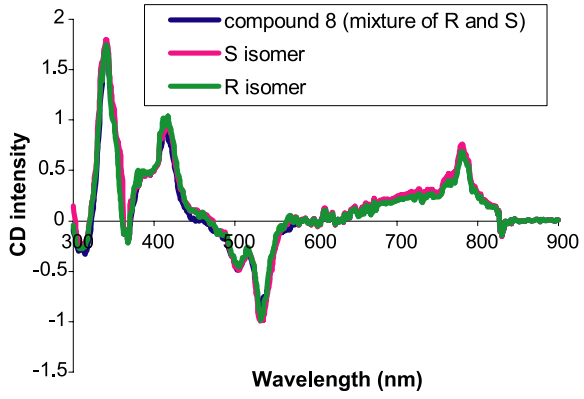


Figure 4. CD spectra of compound **8** and the corresponding *R*- and *S*-isomers.

et band region in bacteriochlorin–HSA complexes were similar to those observed for the pyropheophorbide-a analogs **1** and **2** (Fig. 3). The CD spectra of the *R* and *S* isomers were quite identical. A typical example is shown in Figure 4.

In summary, our results suggest that effective photosensitizers produce specificity towards site II of HSA. However, no direct correlation was observed between the binding constant values and in vivo photosensitizing efficacy. The CD spectra of the photosensitizer–HSA complexes are sensitive enough to show a significant spectral change in the aromatic region. Therefore, CD spectral changes of photosensitizers after interacting with HSA could be used as a valuable screening tool before evaluating new compounds for in vivo PDT efficacy, which will make the screening process more cost effective.

Acknowledgments

The financial support from the NIH (CA55792), Roswell Park Alliance Foundation, and the shared resources of the RPCI support grant (P30CA16056) is highly appreciated.

References and notes

1. Roussel, C.; Del Rio, A.; Pierrot-Sanders, J.; Piras, P.; Vanthuyne, N. *J. Chromatogr. A* **2004**, *1037*, 311.
2. Sudlow, G.; Birkett, D. J.; Wade, D. N. *Mol. Pharmacol.* **1976**, *12*, 1052.
3. Kragh-Hansen, U. *Dan. Med. Bull.* **1990**, *37*, 57.
4. Milanesi, C.; Biolo, R.; Jori, G. *Med. Biol. Environ.* **1988**, *16*, 121.
5. Kessel, D.; Thompson, P.; Saatio, K.; Nantwi, K. *Photochem. Photobiol.* **1987**, *45*, 787.
6. Bertucci, C.; Viegi, A.; Ascoli, G.; Salvadori, P. *Chirality* **1995**, *7*, 57.
7. Zheng, G.; Potter, W. R.; Camacho, S. H.; Missert, J. R.; Wang, G. S.; Bellnier, D. A.; Henderson, B. W.; Rodgers, M. A. J.; Dougherty, T. J.; Pandey, R. K. *J . Med. Chem.* **2001**, *44*, 1540.
8. Chen, Y.; Graham, A.; Potter, W. R.; Morgan, J.; Vaughan, L.; Bellnier, D. A.; Henderson, B. W.; Allan Oseroff, ; Dougherty, T. J.; Pandey, R. K. B. *J. Med. Chem.* **2002**, *45*, 255.
9. Belletti, J.; Ramasubbu, N.; Pandey, R. K.; Dougherty, T. J.; Srikrishnan, T. *Biophys. J.* **1998**, *74*, A367.
10. He, X. M.; Carter, D. C. *Nature* **1992**, *358*, 209.
11. Johnson, W. C. *Annu. Rev. Biophys. Chem.* **1988**, *17*, 145.
12. Murry, R. K.; Granner, D. K.; Mayes, P. A.; Rodwell, V. W. Porphyrins and Bible Pigments. In *Harper Biochemistry*; Appleton & Lange: Norwalk, CT, 1993; pp 337–343.
13. Tsuchida, T.; Zheng, G.; Pandey, R. K.; Potter, W. R.; Bellnier, D. A.; Henderson, B. W.; Kato, H.; Dougherty, T. J. *Photochem. Photobiol.* **1997**, *66*, 224.
14. Li, G.; Graham, A.; Chen, Y.; Dobhal, M. P.; Morgan, J.; Zheng, G.; Kozyrev, A.; Oseroff, A.; Dougherty, T. J.; Pandey, R. K. *J. Med. Chem.* **2003**, *46*, 5349.
15. The CD measurement conditions: HSA was dissolved in pure water. All the chlorins and bacteriochlorins (**1**–**9**) were first dissolved in 0.5–1% Tween 80/water to a concentration of ~400 μM. The concentration was adjusted to 5 μM by diluting it with pure water. Under these conditions, no aggregation was observed. Before measuring the CD spectrum of the complex of the photosensitizer(s) and HSA (in which the final concentrations of both the components are 5 μM), the two solutions were mixed for 1 h to form the corresponding HSA–photosensitizer complex.