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Interaction of cationic chlorin photosensitizers with non-ionic surfactant Tween 80

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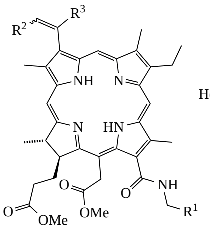
The interaction between cationic chlorophyll photosensitizers (PSs) for antimicrobial photodynamic therapy and non-ionic surfactant Tween 80 as a potential carrier system was studied using absorption and fluorescence spectroscopy. The results, analyzed in terms of our approach and the Stern–Volmer approach, indicate that all three PSs form stable molecular complexes with Tween 80, the PS molecules being located at the periphery of surfactant micelles next to hydrophilic head groups. The higher the charge of the macrocyclic cation, the stronger the interaction of PS with the surfactant.



$R^1 = CH_2NMe_3^+I^-$, $R^2 = R^3 = H$
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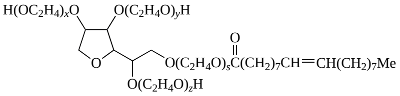


Tween 80

$x + y + z + s = 20$

Keywords: antimicrobial photodynamic therapy, photosensitizer, cationic chlorin, aggregation, non-ionic surfactant, Tween 80, binding constants, fluorescence quenching, carrier system.

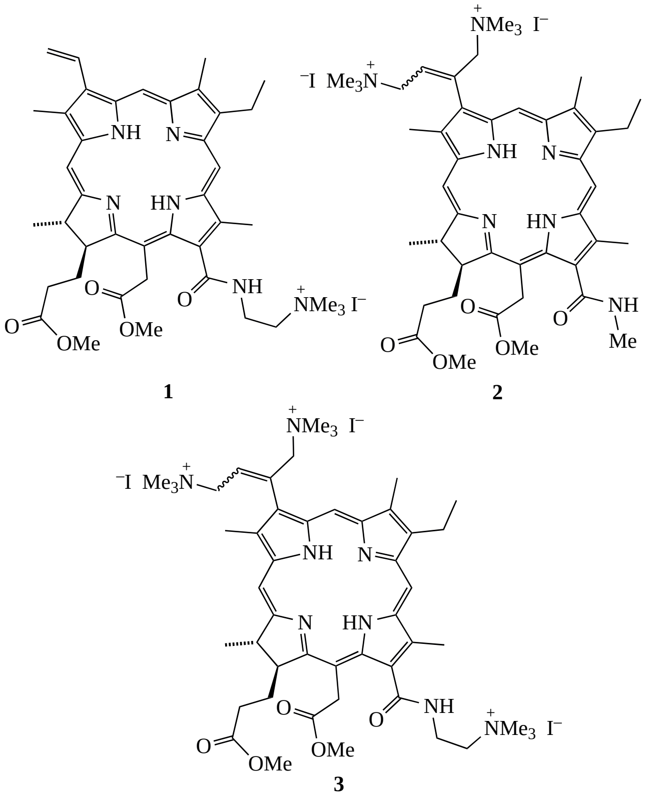
In recent years, antimicrobial photodynamic therapy (APDT) has been regarded as a bright new method of killing many antibiotic-resistant microbes, both *in vitro* and *in vivo*.[1–4] In general, APDT contains three critical components: photosensitizer (PS), visible light and molecular oxygen.[1–3] These non-toxic components, taken together, provide reactive oxygen species that cause deadly damage to bacterial cells. Many of the PSs that are currently being studied as suitable species for PDT are tetrapyrrole pigments,[5–11] similar to those found in natural dyes such as hemoglobin or chlorophyll. Most are capable of effectively killing Gram-positive bacteria, but sometimes have problems with eradicating Gram-negative microbes surrounded by an outer lipopolysaccharide membrane.[3,4] In fact, the attachment of several cationic groups that increase the affinity of PS to the cell wall does not always provide a reliable guarantee of complete photoinactivation of Gram-negative pathogens.[9,12]

It is well known[13–16] that passive drug delivery vehicles, in particular their polymeric, micellar or liposomal forms, significantly improve the solubility, bioavailability and tropism of drugs, including sensitizers for photodynamic therapy. We recently found[9,10,12,15–17] that the addition of low-toxic non-ionic surfactant Tween 80 to the PS formulation significantly increases the solubility of macrocycles in aqueous media, weakens PS aggregation, reduces their dark toxicity to cell lines and seems to enhance the permeability of the outer membrane of Gram-negative bacteria. Fluorescence and spectrophotometric titration studies clearly indicate[16] that water-soluble monocationic chlorin **1** (Figure 1) forms a stable molecular complex with Tween 80, where PS molecules occupy intermediate positions in the surfactant micelle next to polar head groups containing electron-donating oxygen atoms of oxyethylene residues. In this work, we significantly expand this analysis and compare the interaction of Tween 80 with three chlorin PSs **1–3** (see Figure 1) containing one, two or three cationic groups to highlight important features of the interaction of charged macrocycles with non-ionic surfactants.

The synthesis and purification of cationic chlorin PSs **1–3** were carried out as described in Online Supplementary Materials. Spectrophotometric titration of macrocycle solutions with the surfactant was performed at a constant PS concentration (see Online Supplementary Materials). Considering that the accommodation of the PS molecule on the micelle surface affects n neighboring surfactant molecules, and designating the equilibrium concentrations of PS, Tween 80 and their complex as [PS], [T] and $[PS \cdot T_n]$, respectively, we can analyze the results in terms of a simple equilibrium[16] described by the following reaction:

$$[PS] + n[T] \rightleftharpoons [PS \cdot T_n].$$

This approach leads to the equation (1) between the absorbance of the PS solution and the concentration of Tween 80 in the liquid phase, where m_{PS} is the PS concentration equal to 7.3×10^{-6} mol kg^{-1}, m_T^m is the concentration of aggregated Tween 80, which is calculated as the difference between its analytical concentration m_T and its CMC[18,19] equal to 1.2×10^{-5} M, n is the



1

2

3

Figure 1 Molecular structures of the PSs studied: chlorin e_6 13^1-*N*-[2-(trimethylammonio)ethyl]amide 15^2,17^3-dimethyl ester iodide (**1**), 3^1,3^2-bis[(trimethylammonio)methyl] chlorin e_6 13^1-*N*-methylamide 15^2,17^3-dimethyl ester diiodide (**2**), 3^1,3^2-bis[(trimethylammonio)methyl] chlorin e_6 13^1-*N*-[2-(trimethylammonio)ethyl]amide 15^2,17^3-dimethyl ester triiodide (**3**).

number of surfactant molecules in contact with the PS molecule, and, finally, A_0 and $A_{\max}$ are the optical densities of a PS solution in pure water and an aqueous Tween 80 solution, respectively, when the experimental A *vs.* m_T curve reaches a plateau.

$$\begin{aligned} &\lg\{(A - A_0)/[1 - (A - A_0)]\} = \\ &= \lg K_b + n\lg[m_T^m - nm_{PS}(A - A_0)/(A_{\max} - A_0)] \end{aligned} \qquad (1)$$

The model parameters K_b and n were evaluated using the standard iterative mode and are listed in Table 1. The experimental and calculated values for both PSs are compared in Figure 2. We can see that monocationic chlorin **1**, which has one charged group, reveals one mode of binding PS with a surfactant micelle, while PSs containing two and three cationic groups exhibit two different modes. The double-charged chlorin **2** interacts strongly with Tween 80 micelles and binds on average to 1.75 surfactant molecules in the Tween 80 molality range from 0.04 to 0.15 mmol kg^{-1}. In contrast, triple-charged chlorin **3** starts to bind to surfactant micelles at much higher dilutions (see Table 1) and does not reveal such affinity for Tween 80 environment. Comparison of the aggregation number of surfactant (N ~ 58)[19] and the concentration range studied (see Table 1) shows that at reduced concentrations of Tween 80, a significant deficit of micelles is observed in the solution. Consequently, one Tween 80 micelle interacts with several well-hydrated molecules of compound **3**, which can occupy neighboring positions in the micelle structure. This explains the low K_b and n values for this range of surfactant concentrations.

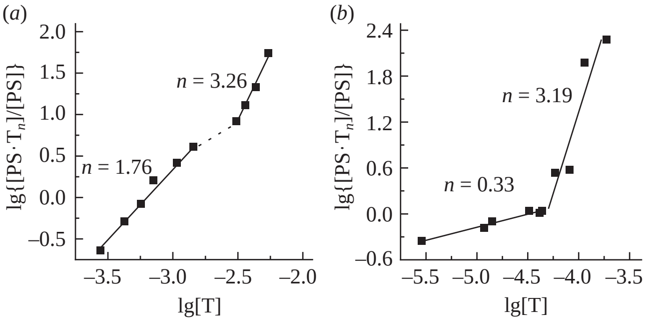


Figure 2 The curves of $\lg\{[PS \cdot T_n]/[PS]\}$ *vs.* equilibrium molality of Tween 80 for (*a*) compound **2** and (*b*) compound **3**. The points are experimental values, and the lines show an approximation using equation (1).

For higher m_T values, another mode appears, which means a significant increase in the interaction of the PS and the surfactant, accompanied by appreciable dehydration of solute species. This effect is more pronounced for compound **3**, which strongly interacts with the surfactant when the PS/micelle ratio approaches unity. Thus, all three cationic PSs exhibit pronounced affinity for Tween 80 micelles, and the corresponding binding constants are very large and rise with increasing cation charge. Perhaps, this is because the interaction of the cationic groups of chlorin with the oxygen atoms of the oxyethylene groups of the surfactant as the main active site strongly contributes to the formation of the PS–Tween 80 molecular complex.

To shed additional light on the interaction of PS and surfactant, we studied fluorescence quenching of their solutions containing both Tween 80 and the well-proven quenching agent potassium iodide. This well-hydrated ionic solute does not penetrate deep into the micelle, which makes it possible to clarify the most probable location of PS molecules on the micelle surface. The fluorescence quenching curves are well described by the Stern–Volmer equation[20] [equation (2)], where F_0 and F are intensities of the PS fluorescence in an aqueous solution of Tween 80 without the quencher and in a solution containing KI, respectively, [I$^-$] is the molality of the quencher, and K_{SV} is the quenching

Table 1 Surfactant concentration ranges, binding constants, stoichiometry of PS–surfactant complex, PS/surfactant molar ratios and Stern–Volmer constants of fluorescence quenching for cationic chlorin PSs **1**–**3** in aqueous solution of Tween 80.

Parameters of equation (1)				Parameters of equation (2)		
m_T/mol kg^{-1}	$\lg K_b$	n	R^2	m_{PS}/m_T	K_{SV}/kg mol^{-1}	R^2
Compound **1**[a]						
$(0.2–5.6)\times10^{-4}$	4.57 ± 0.22[b]	0.93 ± 0.06	0.974	1:60	5.44 ± 0.08	0.987
Compound **2**						
$(3.9–14.6)\times10^{-4}$	5.66 ± 0.29	1.76 ± 0.09	0.994	1:60	7.78 ± 0.08	0.996
$(3.1–5.5)\times10^{-3}$	9.09 ± 0.67	3.26 ± 0.28	0.992	1:6000	2.56 ± 0.03	0.997
Compound **3**						
$(1.6–5.6)\times10^{-5}$	1.44 ± 0.13	0.33 ± 0.03	0.990	1:60	5.40 ± 0.18	0.952
$(0.6–2.2)\times10^{-4}$	14.1 ± 2.9	3.19 ± 0.70	0.935	1:6000	1.33 ± 0.06	0.920

[a] Published[16] data. [b] Uncertainties are standard errors.

constant. The K_{SV} value is easily calculated as the slope of the Stern–Volmer linear dependence (see Table 1 and Online Supplementary Materials).

$$F_0/F = 1 + K_{SV}[I^-] \tag{2}$$

Table 1 indicates that for a PS/surfactant molar ratio of 1 : 60 (one PS molecule per micelle), the quenching constants are rather large and comparable for all three PSs. The higher value for double-charged chlorin **2** is easily explained by the fact that at a PS/surfactant ratio of 1 : 60 ($m_T \sim 7\times10^{-5}$ mol kg^{-1}), a significant part of PS molecules is not yet bound to the micelle surface and is in a hydrated state (see Table 1). When the PS/surfactant molar ratio is equal to 1 : 6000, there is a large excess of Tween 80 micelles in the solution. Thus, the formation of the complex for all cases is completed. Moreover, there is strong evidence that labile Tween 80 micelles interact with each other in such concentrated solutions, which weakens the hydration of the polar head groups.[21,22] This phenomenon should facilitate the binding of PS to the surfactant, which leads to a decrease in the Stern–Volmer constants. Table 1 shows that it is really the case. However, the observed K_{SV} values do not approach zero, which indicates that a small quenching effect still remains. Thus, we can conclude that both double- and triple-charged chlorins located inside the Tween 80 micelle occupy positions next to the hydrophilic head groups and not in the palisade layer around the first carbon atoms of the hydrophobic residue. This finding is in reasonable accordance with chemical intuition and explains the observed K_{SV} values.

In conclusion, we have focused for the first time on the interaction of three chlorin PSs containing one, two or three cationic groups with non-ionic surfactant Tween 80, which is considered a suitable carrier in biological media. Our results clearly indicate that all macrocycles strongly bind to the micelle surface. Both the binding constant and the stoichiometry of the PS–surfactant interaction depend on the nature of the PS and the Tween 80 concentration. In general, the higher the cation charge, the stronger the interaction of PS with the surfactant. Comparison of the fluorescence quenching of solute species in aqueous Tween 80 solutions containing variable amounts of KI leads to the conclusion that polycationic PSs mainly occupy intermediate positions in the Tween 80 micelles next to the hydrophilic ether groups of the surfactant. This seems to be sufficient to ensure the efficient transfer of these potential drugs to appropriate targets in living systems.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2021.01.019.

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