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Photoprocesses of chlorin e6 bound to lysozyme or bovin serum albumin

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ABSTRACT

The ground and excited state processes of chlorin e6 in aqueous solution were studied in the presence of lysozyme and also bovine serum albumin. Non-covalent binding to proteins was analyzed using fluorescence, UV–visible and circular dichroism absorption spectroscopy. The number of binding sites, n, was 1.5–2.4 and the apparent macroscopic dissociation constant, K_d was 0.2–2.5 μM. The binding of chlorin e6 to lysozyme, in contrast to that of bovine serum albumin, imparted fluorescence quenching; circular dichroism spectra revealed a chiral environment upon binding to β-sheets of bovine serum albumin. Time-resolved photolysis showed a longer triplet lifetime upon interaction with the proteins owing to shielding of the dye. The quantum yields for both damage to chlorin e6 (Φ_d) and for protein oxidation (Φ_{ox}) were determined under oxygen-free conditions; Φ_d was smaller because of shielding by the protein with respect to the self-quenching of the free dye. The major effects concerning photooxidation in the absence and presence of oxygen are discussed.

1. Introduction

The primary processes in photosynthesis have been studied in great detail. Many efforts have been made to describe the properties of chlorophyll assemblies [1,2]. The strong electronic interaction of the many chlorophyll molecules in the antenna, however, makes it practically impossible to address a single entity in this large aggregated complex. While monomeric chlorophyll *a* in organic solvents shows both fluorescence and intersystem crossing in substantial quantum yields, the quantum yield of intersystem crossing (Φ_{isc}) of aggregated chlorophyll *a* is small [3]. Another supramolecular aggregate system involves chlorin moieties which play a key role for photosynthesis in green plants and photosynthetic bacteria. The self-assembly of zinc chlorins to an artificial supramolecular light-harvesting device has been intensively investigated [4].

Chlorins are used as sensitizers, their photoredox features are well known and their spectroscopic properties have been investigated widely [5–27]. Chlorin e6 (Chart 1) and chlorin p6 are structurally similar and only the CH_2COO^- group in position 15 is replaced by COO^-, respectively. Some features of chlorin e6 in aqueous solution are known, e.g. Φ_{isc} is large and the fluorescence quantum yield (Φ_f) is moderate. The efficient triplet population is supported by a quantum yield of singlet molecular oxygen production (Φ_Δ) of 0.65 at pH 7–8 [5,8]. The binding conditions of chlorins to proteins have been examined [18–20].

Another class of photosensitizers is constituted by the porphyrin moiety [28–41]. The amount of knowledge about chlorins is much smaller than about porphyrins and only little is available in the literature concerning chlorin-sensitized photooxidation of proteins. The reactive state of porphyrins towards intermolecular processes is generally the triplet, which is efficiently populated, for e.g. *meso*-tetra(4-sulfonatophenyl)porphyrin (TSPP, see Chart 1) Φ_{isc} and Φ_Δ are 0.5–0.6 [35]. Porphyrin and chlorin dyes are suitable as sensitizers for the photooxidation of proteins, such as bovine (BSA) or human (HSA) serum albumin [42,43]. Three dimensional structures, size, shape, and charge distribution of the two frequently applied proteins are well known. However, both BSA and porphyrins may be subjects of aggregation which generally lowers the reactivity [30,31,42]. A variety of other synthetic molecules have been applied for photooxidation of proteins, e.g. TSPP [31–34], chain-substituted pyrenyl peptides [44], methylene blue [45], cyanine dyes [46] and xanthene dyes, such as eosin, erythrosin and rose bengal [47]. The cyanine dyes have attractive spectroscopic and photochemical features but low Φ_{isc} and Φ_Δ.

In this paper we present results on the binding of chlorin e6 in aqueous solution to either lysozyme or BSA and the photosensitized protein oxidation. Lysozyme belongs to the most often studied enzymes [44–46]. We aim at a better understanding of the photoprocesses of chlorin e6, which are induced by binding to the two proteins. Thereby, we elucidate the features and conditions of the photosensitized oxidation of lysozyme and BSA under oxygen-free conditions. The quantum yields of either damage (bleaching, Φ_d) of

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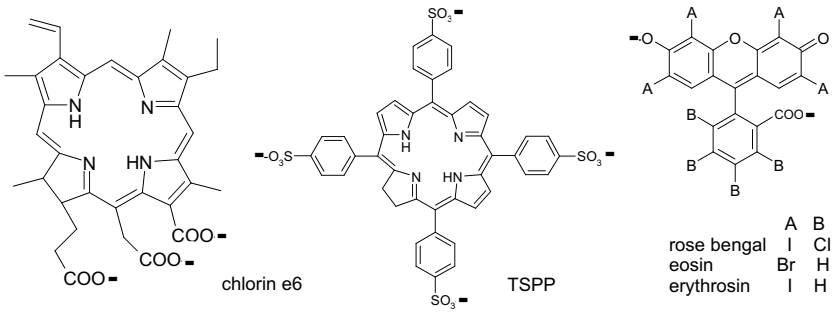


Chart 1.

chlorin e6 or of protein oxidation (Φ_{ox}) in argon-saturated aqueous solution, involving free radicals as key intermediates, were compared with those in the presence of air, involving singlet molecular oxygen as reactant. The photophysical and molecular aspects may provide a basis for related photosensitizer–protein interactions.

2. Materials and methods

Chlorin e6 (Frontier Scientific) [22] and BSA (Sigma) and lysozyme from egg white (Fluka) [46,47] were the same as used previously. Water was from a millipore (milli Q) system. The stock solutions of proteins, typically 100 μM, were freshly prepared without buffer unless indicated otherwise. The dye solutions were also freshly prepared and the concentrations were adjusted to 5–15 μM. The pH was typically 7.6–8.0 and shifted by addition of protons ($HClO_4$) or hydroxyl ions (NaOH). The UV–vis absorption spectra were recorded on a diode array spectrophotometer (HP, 8453). A spectrofluorimeter (Varian Cary, eclipse) was employed to measure the fluorescence spectra. Irradiation was performed with a 250 W Hg lamp and a filter (Schott UG5) or a 1000 W Xe–Hg lamp and a monochromator. The relative quantum yield Φ_d^{rel} of decomposition of dyes in argon-saturated aqueous was obtained from the changes in absorption at λ_a^0 upon continuous irradiation at 380–450 nm. The changes were measured by $(A_0–A_t)/(A_0–A_e)$, where A_t is the absorption at a given time, A_e that at long times and A_0 at the beginning. The relative quantum yield of photooxidation of proteins (Φ_{ox}^{rel}) was obtained by the fluorescence intensity ($\lambda_{ex} = 280$ nm) of the tryptophan residues at $\lambda_f = 350$ nm which decreases vs. the irradiation time [37–39]. A variation of λ_{ex} between 275 and 295 nm did not reveal a significant change in Φ_{ox}^{rel}. As reference we used $\Phi_d = 2 \times 10^{-3}$ for chlorin e6 in air-saturated buffered aqueous solution at pH 7 [5,17]. The CD spectra were recorded using a Jasco J-715 spectrometer with Hamamatsu R376 photomultiplier and appropriate software: Spectra Manager. The flash photolysis operated at $\lambda_{exc} = 308$ nm [22] or at $\lambda_{exc} = 410–690$ nm (Nd-YAG laser + OPO), the absorption signals were measured by a Luzchem system. The measurements were carried out at 24 °C and refer to air-saturated aqueous (for convenience) unless indicated otherwise. Test measurements revealed that for CD and fluorescence essentially the same results were obtained either in air- or argon-saturated aqueous solution in contrast to both steady-state and time-resolved photolysis.

3. Results

The formation of ground state complexes between a dye and a protein leads to changes in fluorescence properties and the UV–vis absorption spectra as well as CD absorption spectra. The various spectral and kinetic features change upon increase of the protein concentration [*P*], keeping the dye concentration [*D*] constant. The results may roughly be distinguished between high loading conditions with many pigments bound to one protein molecule ($[P]/[D] \leq 0.1$) and low loading ($[P]/[D] > 1$) with only one dye molecule per protein or less.

3.1. Absorption properties

The absorption maxima of the Soret and Q band of free chlorin e6 in aqueous solution at pH 7.8 are at $\lambda_a^0 = 405$ and 640 nm. The position of the latter maximum is sensitive to the ionic strength since λ_a is 14 nm red-shifted on addition of 5–10 mM phosphate buffer [22]. The Q band is red-shifted upon increasing of the protein concentration, e.g. to $\lambda_a^P = 667$ nm when bound buffer-free to BSA. Chlorin e6 in concentrations of <10 μM remains monomeric in aqueous solution at low ion strength [7,8]. One or several isosbestic points, e.g. at 520 nm, demonstrate the presence of only two species, free and bound dye. The data are compiled in Table 1 and examples are shown in Fig. 1. No shift in λ_a^P was found for BSA in the presence of 5 mM phosphate buffer, in contrast to lysozyme.

The signal at $\lambda_a^0 = 640$ nm decreases with increasing the [BSA]/[chlorin] ratio and A_{665} increases correspondingly (inset of Fig. 1).

$$n\text{Dye} + \text{Protein} \rightarrow \text{Complex} \quad (1)$$

The effect is described by the characteristic protein/dye concentration ratio for 50% change, $([P]/[D])^a_{1/2}$, which is 0.05–0.2 for BSA and lysozyme (Table 1). Another characteristic parameter is the A^P/A^0 ratio, measuring the change in absorbance at an appropriate wavelength due to low loading. A^P/A^0 is <0.1 for BSA. In the presence of 5 mM phosphate buffer the maximum change in A^P is smaller for BSA and very small for lysozyme, whereas the characteristic $([P]/[D])^a_{1/2}$ ratio remains unchanged for BSA. The

Table 1
Absorption, fluorescence and triplet data of chlorin e6 and data for binding to proteins.[a]

Parameter	No protein	BSA	Lysozyme
λ_a (nm)	405/640 (654)[b]	405/667 (668)	405/667 (654)
λ_f^{ex}(nm)	640	670	640
λ_f^{em}(nm)	650	675	648
A^P/A^0		<0.1 (0.5)[b]	0.5 (0.8)
$([P]/[D])^A_{1/2}$		0.05 (0.2)	0.2
Φ_f^P/Φ_f^0		1.2[c]	0.4[c]
$([P]/[D])^F_{1/2}$		<0.2	0.2
$\Phi_{isc}^P/\Phi_{isc}^0$		0.9	0.3
τ_T (ms)	0.2 [0.2][d]	0.5	0.4

[a] In aqueous solution at pH 7.8 unless indicated otherwise.
[b] Values in parentheses refer to 5 mM phosphate buffer.
[c] Same result using $\lambda_f^{ex} = 380$ or 520 nm.
[d] Value in brackets: pH 4.

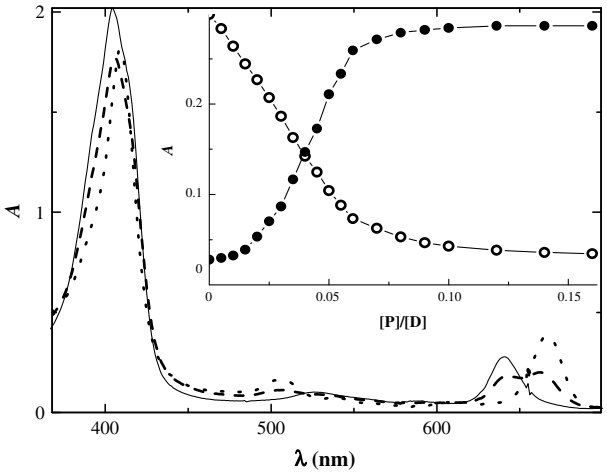


Fig. 1. Absorption spectra of chlorin e6 (14 μM) in air-saturated aqueous solution at pH 7.8 in the absence (full line) and presence of BSA; inset: plot of A_{640} (open) and A_{665} (full) as a function of the BSA concentration.

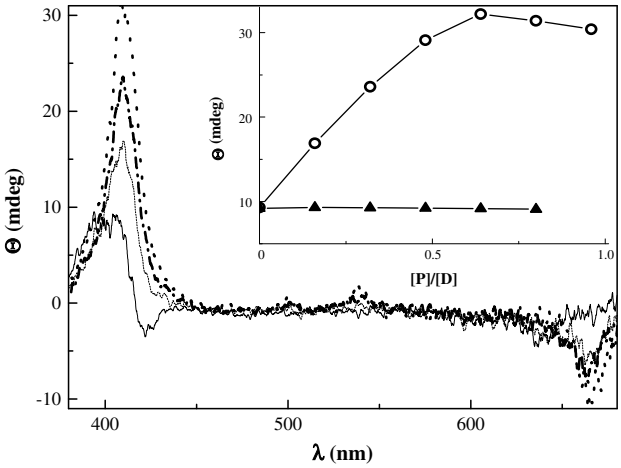


Fig. 2. CD spectra of chlorin e6 (8 μM) in air-saturated aqueous solution at pH 7.8 for 2, 4, 6 and 8 μM BSA; inset: plots of the CD signals at 410 nm as a function of the BSA (○) and lysozyme (▲) concentration.

solution pH of 4 results in protonation of the dye but does not change either λ_a^0 or λ_a^P.

3.2. Binding of chlorin to proteins

The interaction of dyes and proteins is commonly described by the apparent macroscopic dissociation constant (K_d, the inverse binding association constant) and the number (n) of binding sites. For n binding sites the relationship 3, Scatchard plot, is generally used.

$$K_d = [\text{protein}][\text{dye}]^n/[\text{complex}] \quad (2)$$

$$[D_b]/\left([D_f] \times [P]\right) = (n/K_d) - ([D_b]/([P] \times K_d)) \quad (3)$$

Here, $[P]$ is the total protein concentration, $[D_f]$ is the concentration of free dye molecules which are in equilibrium with bound dye molecules $[D_b]$. The dependence of $[D_b]/([D_f] \times [P])$ vs. $[D_b]/[P]$ is in the simple case, i.e. where the binding sites do not interact, a straight line, the intercept/slope gives n and 1/slope is K_d. Scatchard plots for BSA and chlorin e6 in aqueous solution at pH 7.8 have two linear parts (not shown), indicating that two binding sites are present. Data for typical cases are listed in Table 2.

3.3. Circular dichroism

The strong binding of chlorin e6 in aqueous solution at pH 7.8 to BSA is supported by the induced CD spectra shown in Fig. 2 for $[P]/[D] = 1$. In this case the ellipticity (amplitude of the signal at 410 (or 660) nm: Θ) is largest. With increasing the [BSA]/[D] ratio Θ increases, has a maximum at a characteristic dye/protein ratio $([P]/[D])^c_{1/2}$ of ca. 1 and then decreases (inset of Fig. 2). The half-value is $([P]_{1/2} = 3.5$ μM. Virtually no CD signal was found for chlorin/lysozyme, this being in agreement with a very weak interaction.

Table 2
Number of binding sites and dissociation constant of chlorin e6.[a]

Protein	λ_{obs} (nm)	n_1	n_2	K_{d1} (μM)	K_{d2} (μM)
BSA	640	2.0	1.5	0.67	2.4
	667	2.0	1.5	0.78	2.5
Lysozyme	640	2.4	1.8	0.2	0.3

[a] In air-saturated aqueous solution at pH 7.6–8.0.

3.4. Fluorescence properties

The fluorescence emission maximum of chlorin e6 in aqueous solution at pH 7.8 is positioned at $\lambda^f_{em} = 650$ nm. The excitation spectrum is a mirror image of the emission spectrum and the peak is centered at $\lambda^f_{ex} = 640$ nm. The maximum is red-shifted with increasing the BSA concentration, e.g. 25 nm for λ^f_{em} at $[P]/[D] = 1$. Examples of the emission spectra in the absence and presence of BSA are shown in Fig. 3, inset. The data of free and bound chlorin e6 are compiled in Table 1. Lysozyme quenches Φ_f (and the intensity I_f), whereas BSA enhances the fluorescence using either $\lambda^f_{ex} = 380$ or 520 nm. When the Soret band was excited, we found that Φ_f decreases with increasing the protein concentration only for lysozyme, whereas Φ^P_f/Φ^0_f becomes larger, i.e. the ratio of values for $[P]/[D] = 1$ vs. $[P]/[D] = 0$ is $\Phi^P_f/\Phi^0_f = 1.2$. The Q band is not shifted with increasing the lysozyme concentration. The characteristic protein/dye concentration ratio for 50% change, $([P]/[D])^f_{1/2}$, is similar to that obtained on the basis of the absorption spectra.

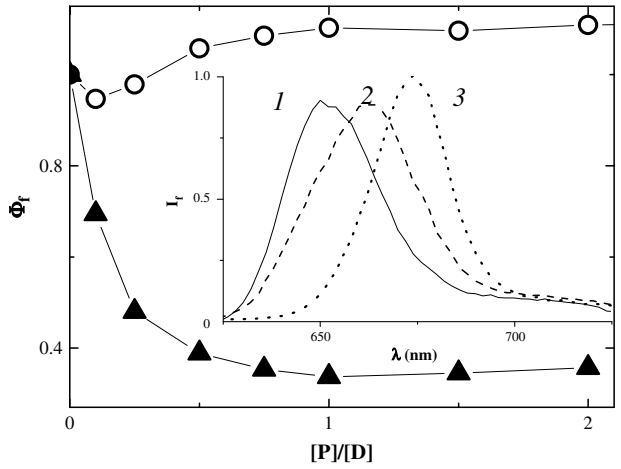


Fig. 3. Plots of Φ_f of chlorin e6 (5 μM, $\lambda^f_{ex} = 520$ nm) in air-saturated aqueous solution at pH 7.8 as a function of the BSA (○) and lysozyme (▲) concentration; inset: fluorescence emission spectra in the absence and presence of 0.5 and 8 μM BSA, 1–3, respectively.

3.5. Transient absorption properties

The triplet state of chlorin e6 has strong bleaching peaks at λ_a = 405 and 640 nm and the decay follows first-order kinetics. The lifetime in air- and argon-saturated aqueous solution at pH 7.8 is τ_T^0 = 3 μs and 0.18 ms, respectively. The rate constant of triplet quenching by oxygen is $k_{ox} = 0.1 \times 10^9$ and 1.5×10^9 M^{-1} s^{-1} in phosphate buffer and ethanol, respectively [22]. The transient difference spectrum of chlorin e6 shows a shift of λ_a and remains otherwise unchanged, when a protein is added, whereas the yield and kinetics are [*P*]/[*D*] dependent. As an example, the spectra are shown in the presence of BSA (Fig. 4). The dependences of $1/\tau_T$ under argon and Φ_{isc} as a function of the [*P*]/[*D*] ratio are shown in Fig. 5a and b, respectively. $\Phi_{isc}^P/\Phi_{isc}^0 = 0.3$ for lysozyme but only a small decrease of Φ_{isc} vs. BSA concentration was found, $\Phi_{isc}^P/\Phi_{isc}^0 = 0.9$ for [*P*]/[*D*] = 0.3–1.

3.6. Photodamage of dye and proteins

Examples of the absorption spectra of chlorin e6 at [BSA]/[*D*] = 0 and 1 upon continuous irradiation at 400–500 nm are shown in Fig. 6. Photodecomposition of the dye was obtained from the changes in absorption at 405 nm. Plots of the normalized absorption decrease as a function of irradiation time are initially linear and the slope is proportional to $1/\Phi_d$. This change is relatively strong for the free dye in argon-saturated aqueous solution. The absorption at λ_a^P in the presence of proteins vs. irradiation time changes similarly. Examples in the absence and presence of BSA are shown in the inset of Fig. 6. The Φ_d values at pH 4 and 7.8 are compiled in Table 3. The photodamage of chlorin e6 under argon is substantial in the absence of proteins, Φ_d is smaller for lysozyme and even smaller for the BSA cases.

Photodamage also takes place for proteins. This photooxidation was observed by fluorescence in the 300–350 nm range. I_f has been reported to be a reliable measure for sensitized inactivation of lysozyme or trypsin [37–39,45]. Results from the latter method are shown in Fig. 7 and Φ_{ox} values are listed in Table 3 for the protein/chlorin systems at [*P*]/[*D*] = 1. Interestingly, the presence of 5 mM phosphate buffer increases most Φ_d values 2–4 fold.

4. Discussion

4.1. Binding of chlorin to proteins

Chlorin e6 at low concentration of 2–20 μM remains monomeric when an organic solvent is replaced by water [7,8]. Pyrrole nitrogens and the two carboxyl groups of chlorin e6 can be protonated, the pK_a = 6.1 and no other inflection point is observable [13]. Addition of proteins to various dyes in aqueous solution results in a marked hypsochromic shift due to stacking of the aromatic residues with those of the pigment. Fetal calf serum shifts the inflection point of chlorin e6 to 7.6 [13]. For chlorin e6 binding to HSA at pH 7

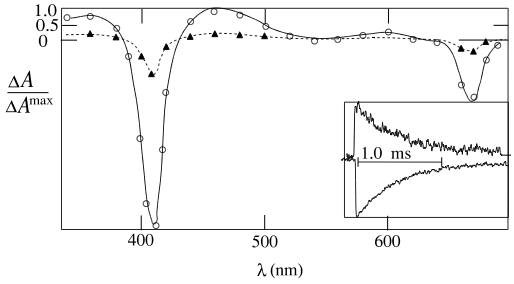


Fig. 4. Transient difference spectra of chlorin e6 (8 μM) in argon-saturated aqueous solution at pH 7.8 in presence of BSA ([*P*]/[*D*] = 1) at 20–90 ns (○) and 1 ms (▲) after the 308 nm pulse; inset: kinetics at 410 (lower) and 460 nm (upper).

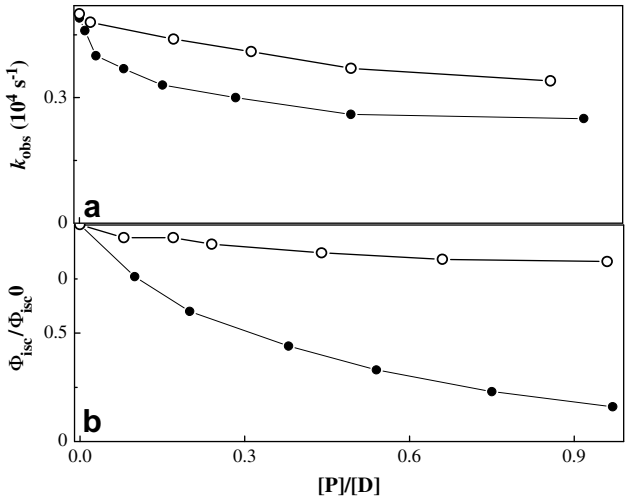


Fig. 5. Plots of (a) $1/\tau_T$ and (b) $\Phi_{isc}^P/\Phi_{isc}^0$ of triplet chlorin e6 (8 μM) in argon-saturated aqueous solution at pH 7.8 as a function of the [*P*]/[*D*] ratio for lysozyme (full) and BSA (open), λ_{exc} = 308 nm.

K_d = 0.05 μM [25] as well as K_d = 13 μM [24] have been reported. This coincides with K_d = 7 μM for binding to BSA [23]. In our hands, K_d is in between these limits and the number of binding sites is n = 1–2; surprisingly K_d of chlorin e6 binding to lysozyme is even lower (Table 2). Biophysical studies on proteins have frequently been performed in solutions as near as possible to the natural situation, e.g. as basic parameters an appropriate buffer and ionic strength or added salt were chosen. However, these salts may cover details of dye–protein interactions and were omitted here. Salt-free conditions were also used to obtain CD spectra in chain-substituted pyrenyl peptides [44] and cyanine dyes [46].

4.2. Effects of proteins on chirality

Free chlorin e6 is achiral and the CD spectra are therefore induced by binding to chiral parts of the proteins. For cyanine dyes left- and right-handedness in CD spectra has been reported for binding to β-sheets of BSA and to α-helices of lysozyme, respectively [46]. Three dimensional structures, size, shape, and charge distribution of the two proteins are well known. BSA exists in the form of a prolate

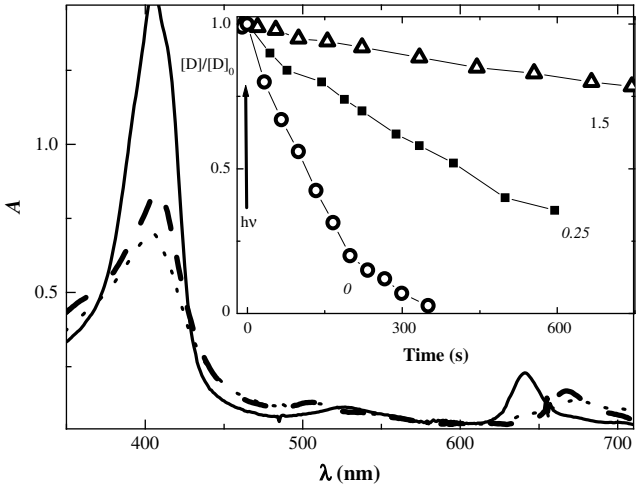


Fig. 6. Absorption spectra of chlorin e6 in argon-saturated aqueous solution at pH 7.8 for 0 (full line) and ca 50% conversion, [BSA]/[chlorin] = 0 (dotted) and 1 (dashed); inset: photodamage, shown as A_{405} vs. the irradiation time [*P*]/[*D*] = 0, 0.25 and 1.5.

Table 3
Quantum yields (x 0.001) of chlorin decomposition and protein oxidation.[a]

		pH	None	BSA	Lysozyme
Φ_d	Argon	4	5	1.8	3
		7.8	3 (2)[b]	0.5 (1.3)	2.5 (3.3)
	Air	4	0.8	5	1
		7.8	0.5 (2.0)[b,c]	3 (5)	0.7 (2)
Φ_{Ox}	Argon	4		0.1	1.0
		7.8		0.15	0.6
	Air	4		0.5	1.4
		7.8		1.4	2

[a] In aqueous solution at $[P]/[D] = 1$.
[b] Values in parentheses: in the presence of 5 mM phosphate buffer.
[c] Taken from Refs. [5,17].

ellipsoid with 185 ions per molecule, and the overall net charge is −18 at pH 7, MW is 66.7 kDa, and the number of amino acid residues is 582. BSA is characterized by three domains and a rigid structure due to seventeen disulfide bonds compared to 6–8 in other proteins; it has 65% α-helices whereas β-motives are absent. The binding of chain-substituted pyrenyl peptides to both BSA and lysozyme causes comparable negative and positive Θ signals [44]. Xanthene dyes bind to specific environments of these proteins and have moderated Θ signals [47]. A similar Θ due to binding of chlorin e6 to BSA was found in contrast to the lysozyme case (Fig. 2). The lysozyme structure is a less flexible ellipsoidal shape, has a prominent cleft that is formed across the face of the enzyme and the content of α-helices exceeds that of the β-sheets (12%).

4.3. Excited state properties

The fluorescence data of chlorin e6 are in line with those in the literature [11,16,17,23,24]. The quantum yield is $\Phi_f = 0.05$ in aqueous solution at pH 7–8. Binding to a macromolecule may decrease or increase Φ_f. For chlorin e6 the fluorescence in the presence of both proteins decreases, $\Phi_f^P = 0.1 \times \Phi_f^0$ at for $[P]/[D] = 1$. This fluorescence quenching could be due to electron transfer, generally being more likely than energy transfer.

The triplet lifetime of chlorin e6 in argon-saturated ethanol is $\tau_T^0 <$ 30 μs and longer than 0.1 ms in phosphate buffered solution, where $\Phi_{isc} = 0.5$ [22]. The triplet data of chlorin e6 in aqueous solution are in line with those of fluorescence; $\Phi_{isc}^P = 0.3 \times \Phi_{isc}^0$ for lysozyme at $[P]/[D] = 1$–2 and is virtually unchanged for BSA (Table 1). In fact, a lower

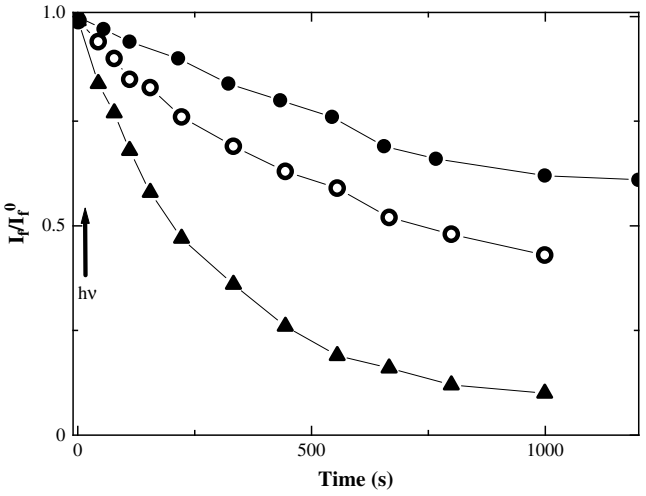


Fig. 7. Photooxidation of lysozyme (full) and BSA (open), shown as I_f ($\lambda_{ex} = 280$ nm, $\lambda_{em} = 350$ nm) vs. the irradiation time, using chlorin e6 (8 μM) in argon-saturated aqueous solution at pH 4 (triangles) and 7.8 (circles) at $[P]/[D] = 1.0$.

Φ_f due to fluorescence quenching by aromatic amino acid components in lysozyme should induce a lower Φ_{isc}. The triplet lifetime of dyes may increase or decrease due to binding to macromolecules. The former could be due to lowering of self-quenching. A value of this rate constant is $k_s = 4 \times 10^7$ M^{-1} s^{-1} for Zn protoporphyrin in ethanol–water [29]. The latter could be due to electron transfer to a protein component. The dependences of $1/\tau_T$ of chlorin e6 under argon as a function of the $[P]/[D]$ ratio (Fig. 5a) clearly shown that shielding takes place for the monomer triplet bound to BSA; the effect is larger for binding to lysozyme.

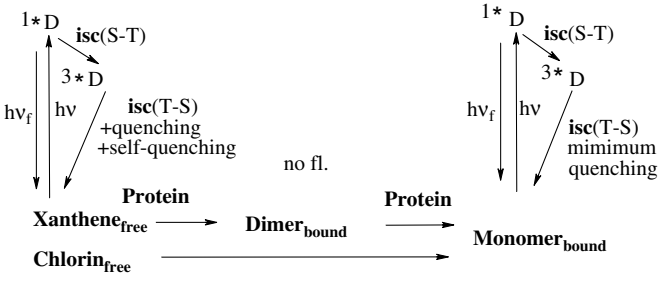
4.4. Photooxidation

The quantum yield of decomposition of chlorin e6 is $\Phi_d = 0.5 \times 10^{-3}$ in air-saturated aqueous solution at pH 7.8 and ten times larger when argon-saturated at pH 4 (Table 3). For mono-L-aspartyl chlorin e6 the quantum yield of oxygen uptake in the presence of 0.5% HSA is 0.07 [8]. The reactive species in the presence of air has been attributed to singlet molecular oxygen [17]. Φ_d of porphyrins is generally lower, a literature value of photodecomposition of TSPP is $\Phi_d = 1 \times 10^{-5}$ [30]. Moreover, the ratio of Φ_d values for chlorin e6 vs. TSPP has been found to be 370 [12], i.e. roughly double of the other literature data [5,17,30]. Quantitative results for chlorins in the presence of lysozyme or BSA could not be found in the literature. The dependence of Φ_d on [BSA] (Fig. 6, inset) is interesting in respect that the damage is lower, when the protein concentration becomes larger. This excludes electron transfer from aromatic amino acid residues of proteins to excited sensitizer states as major effect. Instead, the damage of free chlorin e6 is proposed to be due to self-quenching.

4.5. Comparison with other dyes

It appears attractive to compare the protein/chlorin system with those of xanthene dyes, such as eosin, erythrosin and rose bengal [47] and TSPP [31–34]. The pigments differ markedly by their binding and aggregation potentials in aqueous solution. Φ_d is largest for the flavin mononucleotide, where Φ_{ox} is even larger. Φ_d is smallest for TSPP [30]. For binding of eosin and rose bengal to either lysozyme or BSA the triplet yield is significantly reduced, $\Phi_{isc}^P/\Phi_{isc}^0 =$ 0.2–0.3 for $[P]/[D] = 1$. The major mechanism for xanthene dyes is electron transfer from aromatic amino acid residues of proteins to the sensitizer excited singlet and triplet states. In the cases of flavins, only the sensitizer triplet state is involved. For bound TSPP and chlorin e6 electron transfer from tyrosine/tryptophan residues of lysozyme to the excited singlet state is proposed.

It should be recalled that Φ_d is generally enhanced by binding to a protein [44]. The simplest model contains two molecules, the free monomeric dye and the monomer, which is bound to a protein.



Scheme 1. The large self-quenching efficiency and low triplet quenching for free chlorin and bound as monomer, respectively; for comparison: similar effects were observed for eosin, erythrosin or rose bengal and in addition a non-fluorescent dimer at an intermediate [P]/[D] ratio.

More species have to be considered if aggregation is involved. This has been suggested for xanthene dyes [47], see Scheme 1. For the lysozyme or BSA/chlorin system the two-state model of free and bound monomers appears sufficient, in contrast to cyanine dyes, where non-fluorescent dimers contribute [46].

5. Conclusion

The spectroscopic and photochemical features of chlorin e6 in argon-saturated aqueous solution were studied in the presence of BSA and lysozyme over a large concentration range and at pH 4.0 and 7.8. The relatively low photostability of the free dye is explained by a moderate efficiency of self-quenching. The higher photostability of the bound dye is due to shielding by the protein. No oxidative damage via electron transfer from aromatic amino acid constituents and the triplet state of chlorin e6 takes place. High loading to lysozyme, however, quenches the excited singlet state, an effect which is virtually absent for BSA.

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