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Short-Wavelength Absorbing Complexes of Chlorophyll *a* in Micellar Solution of Cationic Detergents

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Chlorophyll, Micelles, Detergent, Aggregation, Absorption, Circular Dichroism, Molecular Orbital

Micellar aggregates have been prepared from chlorophyll *a*. Whereas most detergents including non-ionic, zwitterionic and anionic ones, produce aggregates with long-wavelength shifted and enhanced Q_Y absorption, the cationic detergents hexadecyl-trimethylammonium-bromide and hexadecyl-pyridiniumchloride produce complexes with opposite traits: The Q_Y absorption is shifted to shorter wavelengths, and it is hypochromic. The optical activity is increased as in the long-wavelength shifted complexes, but the cd bands are non-conservative and coincide with the absorption maxima. The complexes are most likely small aggregates of chlorophyll with a changed geometry as compared to the "standard" ones.

Introduction

Micellar complexes of chlorophylls and bacteriochlorophylls have gained attention due to the spectroscopic similarities with (bacterio)chlorophyll proteins containing the same pigments [1–8]. It has been argued that these similar properties result from the formation of aggregates with similar geometry, reflecting an inherent property of the large aromatic systems, *e.g.* chlorophyll. This view has been strengthened recently: Very similar types of aggregates have been found i) in the primary donor P960 or P870 of bacterial reaction centers as determined by X-ray crystallography [9–11], ii) *in vacuo* as calculated by molecular orbital methods [12], and iii) in aggregates formed in

mixed organic-aqueous solvent systems as determined by the analysis of optical spectra [2, 4].

Exciton theory principally predicts both red- and blue-shifted spectra depending on the relative orientation of the monomers in chlorophyll aggregates, and concomitant hyper- und hypochromism [see 2, 4]. All chlorophyll proteins and *in vitro* chlorophyll aggregates investigated to date exhibit nonetheless a red-shifted Q_Y band. Here, we wish to report the formation of blue-shifted non-covalently linked aggregates of chlorophyll *a* with hypochromic Q_Y band, which are formed in micellar solutions of cationic detergents.

Materials and Methods

Chl *a* was extracted from spray-dried *Spirulina geitleri* and purified by dioxan precipitation [13] and subsequent chromatography on DEAE-cellulose [14]. The pigment was deposited on the walls of plastic vials by evaporating an ethereal solution in a stream of nitrogen, and then redissolved in detergent solution (0.1% in tris buffer, 10 mM, pH 7.5, if not stated otherwise) with sonification (Branson model 220) until the spectra remained constant. The final solutions had an optical density between 0.5 and 1 cm^{-1} in the red spectral band, corresponding to Chl *a* concentrations of 10–20 μM . The position of the near-infrared band of the complexes was not affected by the concentration in this range.

The following detergents were used: CTAB (hexadecyl-trimethylammoniumbromide), CPC (hexadecyl-pyridiniumchloride), LiDS (Lithium dodecylsulfate), and Triton X-100 (Octylphenoxy-polyoxyethylen-glycol) from Serva, Heidelberg; RDL 203/S (dodecanoic acid-ethanolamid), Brij 30 (Polyoxyethyleneglycol-monododecyl-ether), R-AM/V (N-3-hydroxyethyl-N-carboxymethylfatty acid amidoethylamin, Na-salt), R-AM/2L (N-3-hydroxyethyl-N-carboxymethyl fatty acid ethylamin, Na-salt), R-IPL-203 (dodecanoic acid-isopropanolamid), R-B204 (Alkylamidopropyl-dimethylaminoxid), all from Rewo, Steinau; Triton-H66 (Alkylphosphatester, Na-salt), Triton-QS44 (Alkylphosphatester), from Bayrol, München. All detergents were used without further purification.

Absorption spectra were recorded in a model 320 spectrophotometer (Perkin-Elmer), cd spectra

Abbreviations: Chl, chlorophyll; Bchl, bacteriochlorophyll; CTAB, hexadecyl-trimethylammoniumbromide; CPC, hexadecyl-pyridiniumchloride.

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in a dichrograph model V (Jobin-Yvon), and fluorescence spectra in a fluorolog system (Spex). The fluorescence spectra are uncorrected.

Results

The absorption maxima for the complexes formed with the different detergents are listed in Table I. With most of the detergents, chlorophylls form two types of complexes. One has absorptions similar to chlorophyll in monomeric solution, *e.g.* in methanol, the other one absorbs around 740 nm in the red spectral region. A typical example is shown in Fig. 1. The oscillator strength of the Q_Y band of the latter is enhanced about two-fold. The optical activity is increased by orders of magnitude, but the s-shaped cd-band is nearly conservative (not shown). The ratio of the two forms depends on the detergent concentration, with the 740 nm absorbing form favored at lower concentration, but is also dependent on the structure of the detergent used. Red-shifted complexes are also formed with pheophytin a (λ_{\max} around 700 nm, not shown). These spectral traits and concentration dependencies are very similar to the situation found with bacteriochlorophylls [1–8].

A different situation is observed with the two cationic detergents, CTAB and CPC. Again two

Table I. Absorption maxima of micellar complexes of chlorophyll *a* with several detergents of different structural types. See Materials and Methods for structures. All detergents were 0.1% except for the cationic ones (CTAB and CPC) which were 0.05%. Complexes marked with “++” are essentially devoid of monomers absorbing around 665 nm, the ones marked with “+” give mixtures (see *e.g.* Fig. 1).

Detergent	Type	Complex formation	Absorption maximum [nm]
Triton X-100	non-ionic	++	742
RDL 203/S	non-ionic	++	742
R-IPL-203	non-ionic	++	742
Brij 30	non-ionic	+	744
R-B204	non-ionic	+	742
Triton H 66	anionic	+	742
Triton QS44	anionic	++	742
CPC	kationic	+	646
CTAB	kationic	+	646
R-AM/V	zwitterionic*	+	742
R-AM/2L	zwitterionic*	++	740

* Amphoteric between pH 4 and pH 7.

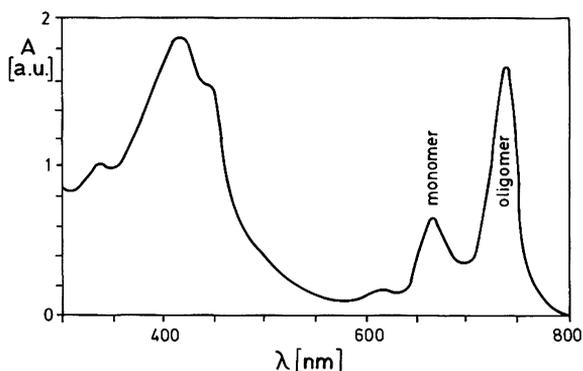


Fig. 1. Absorption spectrum of a micellar solution of chlorophyll *a* with Triton X-100 (0.1%) in Tris buffer (10 M, pH 7.5).

complexes are formed, one of which absorbs like chlorophyll in monomeric solution. The second complex formed absorbs at 646 nm (Fig. 2),

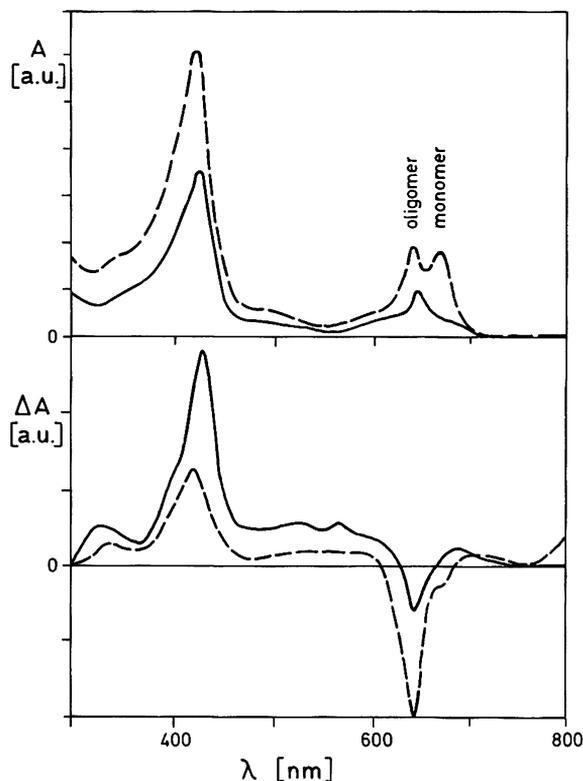


Fig. 2. Circular dichroism (bottom) and absorption spectra (top) of micellar solutions of chlorophyll *a* with hexadecyl-trimethylammoniumbromide in Tris buffer (10 mM, pH 7.5). Detergent concentration 0.008% (—) and 0.05% (---).

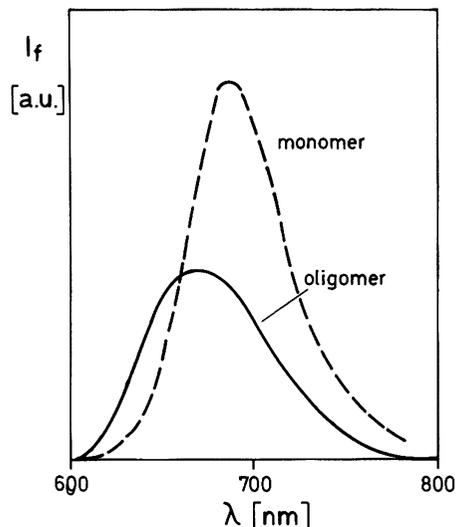


Fig. 3. Fluorescence spectra of a micellar solution of chlorophyll *a* with hexadecyl-trimethylammonium-bromide (0.05% in Tris buffer, 10 mM, pH 7.5) excited at 400 (—) and 450 nm (---). The absorption spectrum of this solution is shown in Fig. 2, top, dashed line.

however, which corresponds to a blue-shift of 20–22 nm as compared to *e.g.* methanolic solution. The Soret band is blue-shifted as well to 420 nm. The oscillator strength of the Q_Y band is decreased, and it is strongly optically active showing essentially a purely negative band peaking at the wavelength of maximum absorption (Fig. 2). The complex shows moderate fluorescence, and the emission band is blue-shifted as well (Fig. 3). As in the other detergents, the ratio of the two complexes depends on the detergent concentration, with the short-wavelength form dominant at low concentrations. At 0.008% CTAB, the blue-shifted form is essentially pure (Fig. 2), but already at .05% there is an equal absorption of the two species at 642 and 668 nm. The short-wavelength absorbing complex is also unstable towards detergents forming red-shifted complexes by themselves. As an example, the addition of LiDS (0.5%) shifts the absorption to 658 nm, and higher concentrations give a mixture of two forms absorbing at 672 and 746 nm, which is similar to the situation with LiDS alone.

Discussion

The red-shifted complexes formed with Bchl and Chl alike have been related to aggregation of

the pigments (see [5, 8]). In detergent solution, aggregation numbers of the pigments are small. Using exciton theory, Scherz and Parson [2, 4] suggested a dimeric structure of the basic unit in which the two macrocycles are nearly parallel ($\approx 15^\circ$ angle between the planes of the macrocycles) at a distance of $\approx 3.5 \text{ \AA}$, and in which only part of the macrocyclic system overlaps due to a lateral displacement of both macrocycles from the center of the dimer. The Q_Y axes form an angle of $\approx 145^\circ$.

It is surprising that this structure, which is formed in micelles of a variety of detergents, and also in mixed aqueous-organic solvent systems, closely resembles the structure of the Bchl-special pair in reaction centers of *Rhodospseudomonas viridis* and *Rhodobacter sphaeroides*, *e.g.* the primary donor involved in light-induced charge separation [9–11]. Moreover, a similar arrangement has been calculated by Plato *et al.* [12] as the minimum energy arrangement in the absence of interactions with the solvent. These similar arrangement of the two chlorophylls in very different environments – vacuum, detergent micelle, pigment micelle, or protein – points to an inherent tendency of the bacteriochlorophyll molecule in forming aggregates of this special geometry.

The present study was undertaken in part to test if the same type of complex can be formed as well with chlorophyll *a*. This was indeed observed and further strengthens the hypothesis. Nearly independent of the detergent used, there is always the formation the same type of red-shifted complex as shown in Fig. 1 for Triton X-100, if judged from the similarities of their absorption and cd spectra. This is true but for two exceptions, *viz.* the cationic detergents CPC and CTAB, which form blue-shifted complexes. It is principally inherent to exciton theory, that both blue and red shifts (as well as none at all) can occur in dimers (or higher aggregates) of chlorophylls, depending on the relative orientation and distance of the two pigment molecules (see *e.g.* [2]). The present example is to our knowledge nonetheless the first case among the many different aggregates of chlorophylls formed by association or a single covalent linkage, in which a blue-shifted species has actually been observed. A blue-shifted bis-chlorophyll-cyclophane has been reported, however, by Overfield *et al.* [16] in which the two pigments show little interaction.

Bchl *a* complexes have been studied before with the same two cationic detergents [6]. Generally, the latter are strongly dissociating both to long-wavelength absorbing complexes of Bchl *a* with other detergents, and to Bchl *a* proteins. In our hands, of the two detergents, only CTAB does form a small proportion of Bchl *a* aggregates (besides monomers) at all at low detergent concentrations. However, these complexes are red-shifted similar to the ones observed in any of the other non-ionic, anionic and zwitterionic detergents studied. This points to a somewhat different aggregation pattern in the

Bchl as compared to the Chl chromophore, which may be related to the different oxidation states of the macrocycles, or the different substitution pattern. Differences of this kind should be important in the interactions of polypeptide and pigments in (bacterio)chlorophyll proteins as well.

Acknowledgements

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