Long-wavelength-absorbing forms of bacteriochlorophyll a in solutions of Triton X-100

(photosynthesis/circular dichroism/chlorophyll micelle/chlorophyll aggregation)

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ABSTRACT

At least three forms of Triton X-100-solubilized bacteriochlorophyll a (BChl a) have been characterized by UV/visible/near-IR absorption and CD spectra. One, absorbing at 770 nm, is similar to a monomeric solution in methanol. The two others have strongly red-shifted absorption peaks (860 nm and 930, 835 nm) and intense complex CD bands in this region, indicative of strong interaction of at least two and three molecules of BChl a, respectively.

The understanding of bacterial photosynthesis has greatly advanced with the isolation and characterization of well-defined complexes of bacteriochlorophyll a (BChl a, I) with proteins (1–4). A peculiar property of all such complexes is a more or less pronounced red shift of the BChl a near-IR absorption band—e.g., to 870 nm for the primary donor in reaction centers (Fig. 1) as compared with 770 nm for the same BChl a in methanol solution (Fig. 2).

The red shifts of these BChl a–protein complexes (as well as similar shifts in chlorophyll a–protein complexes) are as yet only partly understood and subject to much theoretical, spectroscopic, and synthetic work. They have been related to aggregation (5–14) or hydration (or both) (5, 6, 12–15), enolization of the chlorophyll groups (16–19), point charges in their environment (20), and charge-transfer complexes (21). In particular, the primary donor P870 in BChl a-containing reaction centers has been suggested to be a 'special pair' of BChl a, with their exact relationship still under discussion (22–27).

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Triton X-100 (II, m = 9 or 10) is next to dodecyltrimethylammonium bromide the most commonly used detergent for the isolation of bacterial reaction center and light-harvesting complexes.

II

We noticed, during the isolation of reaction centers from *Rhodospirillum rubrum* G9 and *Rhodopseudomonas spheroides* R26 with Triton X-100, fractions that looked superficially like reaction centers contaminated with solubilized BChl a and bacteriopheophytin a. The 805-nm band was, however, small or even absent, and the 570-nm band was somewhat blue shifted and did not show any reversible bleaching. By systematic solubilization studies of BChl a with detergents, we have now shown that Triton X-100 induces strongly red-shifted absorption peaks in solubilized BChl a, with maxima around 830, 860, and 930 nm, and we here present our results on the BChl a species present in these solutions.

EXPERIMENTAL

*Rs. rubrum* G9 and *Rp. spheroides* R26 were grown in Hutner's medium (28) at 27°C. BChl a was isolated by a modification of the method of Sato and Murata (29), which principally involves solvent extraction, precipitation of BChl a as a dioxane complex (30), and chromatography on DEAE-cellulose (29). The chromatography was alternatively also done on sugar columns (30). Triton X-100 was purchased from Serva (Heidelberg, Federal Republic of Germany) and used as received. All solvents were distilled or purified over alumina (Woelm, Eschwege, Federal Republic of Germany) prior to use. UV/visible/near-IR absorption spectra were recorded on a DMR 22 (Zeiss, Toberkochen, Federal Republic of Germany) spectrophotometer equipped for cross-illumination with suitable interference and cutoff filters. CD spectra were recorded with a dichrograph Mark V (Yvon-Jobin, Longjumeau, France) equipped with a red-sensitive photomultiplier. Centrifugation was done in a Hitachi (Japan) model 85P preparative ultracentrifuge. For the detergent-solubilization experiments, BChl a was distributed on the walls of a small glass vessel (3–5 ml) by drying an ethanol or a methylene chloride solution (1 ml) containing about 0.1 mg of the pigment with a stream of nitrogen. The vessel was then filled with N2-saturated 10 mM Tris buffer (pH 7.5), or water, containing Triton X-100 (0.1% (1.08 mM) unless indicated otherwise.

Abbreviations: BChl a, bacteriochlorophyll a, CTAB, cetyltrimethylammonium bromide.

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wise]. In one series of experiments, the mixture was kept without agitation at ambient temperature. In another series, it was agitated in an ultrasonic bath (Branson 220). Molar extinction coefficients (ε) and ellipticities (θ) are throughout determined with respect to the diethyl ether solution (31). For determination in other solvents and in cetyltrimethylammonium bromide (CTAB) solution (1%), an ethereal solution of BChl a of known absorption was dried down in a stream of N₂ and redissolved in the same volume of the solvent of interest. For determination of ε and θ in the Triton X-100-solubilized BChl a species, the latter were treated with 5% CTAB. The extinction coefficients of the resulting solutions (λ_{max} = 770 nm), containing both Triton X-100 and CTAB, were assumed to be identical to those of a solution in 1% CTAB alone.

RESULTS

Absorption Spectra. Absorption spectroscopy of the supernatant in the unagitated vessels showed a slow dissolution of BChl a into the detergent, with a long-wavelength absorption maximum at 770 nm. The long-wavelength absorption of this form is essentially identical to that of a solution of BChl a in methanol (Fig. 2). Within hours, additional bands emerge around 830–960 and 930 nm. The relative intensities of the red-shifted bands vary among samples in a yet incompletely understood way.

Generally, there is only one red-shifted band (860 nm) at early stages of the dissolution process. It can be enriched by centrifugation (225,000 × g) in a sucrose density gradient (0–1 M) for 1.5 hr. Fig. 3 shows a spectrum of the sharp zone focused at r = 1.105 g/cm³, with the lighter fractions being uniformly and only weakly colored and containing predominantly the short-wavelength form absorbing around 770 nm.

Fractions enriched by gradient centrifugation in the 860-nm form are unstable and develop an absorption peak around 930 nm. This shift is accompanied by a gradual blue shift of the band positioned originally at 860 nm to about 835 nm. This indicates that the 860-nm form actually transforms into a form(s) absorbing around 835 and 930 nm, an interpretation supported by the CD spectra discussed below and by the constant ratio of the two bands. This form can be obtained best in a rather pure form if the BChl a is solubilized by sonication. A reproducible preparation (Fig. 4) involves sonication of 30–60 μg of BChl a per ml of the final solution for 30 min in aqueous or Tris-buffered Triton X-100 (0.1%). Again, the 770-nm band is most pronounced early in the process but eventually replaced by bands at 835 and 930 nm. The ratio of these two bands remains fairly constant (1.25–1.35) during the entire process.

The long-wavelength-absorbing forms of Triton X-100-solubilized BChl a are partly transformed back to the 770-nm form by the addition of a large excess Triton X-100 (5%) and can be fully transformed to the 770-nm form by the addition of 1–5% cationic detergent such as CTAB. This effect has been used to determine the extinction coefficients of the long-wavelength forms shown in Figs. 3 and 4.

CD Spectra. The formation of long-wavelength absorbing-forms is accompanied by the development of very intense long-wavelength bands in the CD spectrum. Their extremely high ellipticities (θ ≈ 10⁵) allow their detection at early stages of the solubilization process, when the 770-nm form (θ = 5 × 10⁶) is still present in large excess. The short-wavelength (770-nm) form of the Triton X-100-solubilized pigment has a CD spectrum that is again similar to that of a methanolic solution. The latter exhibits only a single positive weak band (θ_{max} = 4,500) in the red spectral region (Fig. 2). The red-shifted forms have, by contrast, complex bands of much higher ellipticities. The near-IR band of the 860-nm form is S-shaped and narrow (40 nm be-
between the extrema, with the crossing of the baseline ($\lambda = 874$ nm) at the long-wavelength side of the maximum of absorption. Its intensity is increased by more than 2 orders of magnitude as compared with the 770-nm form. An approximate molar ellipticity scale (Figs. 3 and 4) has been obtained from the extinction coefficients. S-shaped CD bands also occur as well in the 600-nm ($Q_0$ band) and the near-UV (Soret) range.

The CD spectrum of the form(s) absorbing at about 835 and 930 nm is even more complex, with three extrema in the near-IR range (Fig. 4). The molar ellipticities are again more than 2 orders of magnitude higher than for the methanol-solvent solution (Fig. 2). There is no indication that the spectrum in Fig. 4 is a superposition of the 860-nm form with the spectrum of other form(s) but the absence of shoulders suggests that the 860-nm form is to a large extent transformed into new species.

DISCUSSION

The CD spectra of the long-wavelength forms observed here are conservative—e.g., they sum to zero—which is indicative of strong exciton coupling among BChl a molecules (32). The 880-nm form has two and the 930/935-nm form has three maxima, which would suggest the close proximity of (at least) two and three pigments, respectively. Aggregation has often been invoked to explain the long-wavelength shifts in various BChl a forms, including BChl a-protein complexes (see, e.g., ref. 6). Shifts of this magnitude have hitherto not been observed, however, in covalently linked dimers aimed to simulate the properties of the primary donor P870 in the reaction center complex (10, 26). Protein-free BChl a forms absorbing at long wavelengths have been observed in methanol/water and acetone/water mixtures (5, 34-37), in (hydrated) BChl a microcrystals (38, 39), and in BChl a films (35, 40). They have been interpreted in terms of aggregation of hydrated BChl a, which is supported by theoretical studies (41). There are two more-detailed studies concerning the formation of different BChl a forms in solution and their interconversion. One reports a series of BChl a hydrates that are believed to be aggregates linked by water molecules but in different ways (5). Some of the absorb-

The report concerns BChl a species in acetone/water mixtures. Komen (34) reported in 1956 two different long-wavelength species, one absorbing around 860 nm in a 45:55 mixture and the other absorbing at 830 and 930 nm in a 1:9 mixture. There is a remarkable similarity in the absorptions between these forms and the ones formed in Triton X-100, including a similar ratio of the two bands in the 830/930-nm form.

When repeating Komen’s preparations, we obtained further support that the two systems may be quite similar (data not shown). First, the 860-nm form is rather unstable, too, in the acetone/water mixture. The (turbid) solution transforms on standing to the 830/930-nm form. Second, the CD spectrum of the 830/930-nm form is almost identical to that of the 835/930-nm form in Triton X-100 (the CD spectrum of the 860-nm form in acetone/water is uninterpretable due to interference caused by its turbidity).

The detergent complexes described here are formed readily in Triton X-100. At concentrations of 0.5–1% at 20°C in water, Triton X-100 forms micelles containing about 38 molecules of detergent, with an inner core of 50 Å diameter (ref. 42 and refs. cited therein). If one assumes (i) a similar micelle in the 0.1% solutions we used and (ii) no significant change in the micelles due to the incorporation of BChl a, a ratio of 0.05 to a maximum of 1 molecule of BChl a per micelle is obtained for the unsolicited solutions, containing mostly the 770-nm form. If the pigment were equally distributed among the micelles, this would indicate monomeric solubilization of BChl a at the beginning or at low concentrations of BChl a. The solutions enriched in the 835/930-nm form formed by prolonged standing and especially by sonication have a BChl a micelle ratio of 1.5–4. Assuming again an equal distribution, this suggests a rather small number of BChl a molecules per micelle, compatible with the minimum number of strongly interacting BChl a molecules as derived from the CD data. Taken together, these results are compatible with a process in which at first monomeric ($\lambda_{max} = 770$ nm) and then di- (860 nm) or trimeric (835, 930 nm) clusters of BChl a are solubilized in the Triton X-100 micelles. This is also corroborated by the second of the 835/930-nm form (n ≫ 3) to the 770-nm form by addition of 5% Triton X-100 to the former (data not shown). One further structural detail is the presence of only one extra ligand at the central Mg atom in all three forms of BChl a micellar solutions. This is inferred from the short ($\lambda = 580$ nm) absorption of the $Q_0$ band, which is characteristic of a pentacoordinated central Mg in BChl a (43, 44).

Complexes with similar absorption are formed also with some other detergents. The Triton X-100 complexes absorbing at long wavelengths are unstable, however, to common ionic detergents such as sodium dodecyl sulfate and, especially, dodecyltrimethylammonium bromide at moderate concentrations (1–5%), which transform them quantitatively to a form absorbing like BChl a in solution around 770 nm. This reversible transformation proves at the same time that the absorption changes observed during the formation and transformation of the BChl a forms are not due to chemical reaction. In fact, 13'-hydroxy-BChl a, which is a common contaminant and reaction product with spectrum similar to that of BChl a (45) does not form such long-wavelength forms.

Long-wavelength-absorbing detergent complexes have been observed, too, with the plant pigment chlorophyll a (46–49) and related to chlorophyll a hydrates (15, 50). They are formed, however, among others with just the detergent sodium dodecyl sulfate, which dissociates the Triton X-100 complex of BChl a.
This different influence of detergents on BChl a and chlorophyll a complexes indicates a somewhat different structure.

Finally, it should be pointed out that there is a remarkable coincidence not only between the absorption but also between the CD spectra of the BChl a–Triton X-100 complexes and those of the BChl a–protein complexes. Most of the bacterial light-harvesting BChl a–protein complexes (see, e.g., refs. 51–53) give S-shaped CD bands of similar ellipticity and the same or opposite sign as the 860-nm form shown in Fig. 3, and the CD spectrum of the 835/930-nm form (Fig. 4) is similar, although red shifted, to the BChl a-derived part (λ = 790 nm) of the CD spectrum from bacterial reaction centers (Fig. 1; see also ref. 38).

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