Antioxidant effects of carotenoids in a model pigment–protein complex

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INTRODUCTION

The photosynthetic apparatus in purple photosynthetic bacteria comprises reaction centers and light harvesting pigment protein complexes LH1 and LH2. The latter, i.e. the peripheral antenna complex, is not produced by all bacterial species. The crystal structures of LH2 complexes from several species of phototrophic bacteria were solved with a high resolution (McDermott et al., 1995; Koepke et al., 1996) while the structure of LH1 is known in less detail. As shown by Ghosh et al. (1988) each complex is composed of identical blocks of low molecular weight and very hydrophobic α- and β-apoproteins to which bacteriochlorophylls (BChls) and carotenoids (Crts) are non-covalently attached (Cogdell et al., 1996). In the absence of Crt, the pigment-polypeptide units spontaneously oligomerize to form the complex whose main absorption maximum is located near 870 nm. In the presence of Crt this band shifts to 880 nm (in the case of Rhodospirillum rubrum). In micellar media, the Crt-less LH1 (B870) reversibly dissociates into monomeric subunits (Loach et al., 1995; Fiedor et al., 2009). The ability of B870 to undergo reversible dissociation renders the complex a unique model system that allows for investigation of interactions occurring between its components. The oligomerization and reconstitution of LH1 from its native or chemically modified components has been studied extensively (Davidson et al., 1981; Miller et al., 1987; Strugis et al., 1994; Frank, 1999; Pandit et al., 2001). Also a full reconstitution, including all components of the LH1 antenna was achieved (Davis et al., 1995; Fiedor et al., 2001b; Fiedor et al., 2004; Fiedor et al., 2009).

It has been shown that BChl components of LH1 complexes undergo oxidation upon treatment with potassium ferricyanide. Such treatment leads to changes in the absorption, circular dichroism (CD), fluorescence emission, and electron paramagnetic resonance spectral signals of the complex (Gomez et al., 1982; Picorel et al., 1984; Law et al., 1998). We have previously studied the effects of Crt on stability of BChla and its derivatives in a model system (Fiedor et al., 2001a; Fiedor et al., 2002), however, so far, the effect of Crt on stability of LH1 complexes exposed to chemical oxidation has not been demonstrated. Crts are structurally and functionally a very diverse group of isoprenoid pigments (Britton, 1995). They occur in all photosynthetic organisms in which they carry out various functions, with light harvesting and (photo)protection considered as the most important. Crts act both as physical quenchers of (B)Chl excited states and efficient scavengers of reactive oxygen species (ROS). They also act as chemical quenchers of ROS undergoing irreversible modifications that may be responsible for a change from the antioxidant to prooxidant activity (Fiedor et al., 2001a; Fiedor et al., 2005).

In the present paper, we demonstrate our results on the role of Crt in stabilization of pigment-protein complexes against chemical oxidation. The model Crt-containing and Crt-less LH1 complexes were subjected to chemical oxidation with hydrogen peroxide or potassium ferricyanide. The antioxidant and stabilizing activity of Crt was monitored by following the changes in the electronic absorption and fluorescence emission spectra of the oxidized complexes.

MATERIALS AND METHODS

Isolation of spheroidene. Spheroidene (Sph) was isolated from the cells of Rhodobacter sphaeroides 2.4.1 as described in Fujii et al. (1998) with minor modifications. The purity of the isolated Sph was checked spectrophotometrically and by HPLC (Fiedor, 2007).

Preparation of B870. Crt-depleted LH1 was prepared as described by Fiedor et al. (2004; 2009) and used immediately after preparation.
Reconstitution assay. The incorporation of Sph into LH complexes was carried out as described previously (Fiedor et al., 2004). Briefly, for the reconstitution, a portion of bacterial Crt-less LH1 in 0.033% LDAO was titrated with a solution of Sph in acetone (OD at 280 nm ~1.2). The progress of the reconstitution was monitored by absorption spectroscopy. The reconstitution was considered complete when a red shift of the LH1 Qx band from 873 nm to 880 nm was observed. The freshly prepared Sph-binding LH1 (Sph-LH1) was kept overnight at 4°C and used without further purification.

Chemical stability of B870 and Sph-LH1. The resistance of Crt-less and Sph-containing LH1 complexes toward chemical oxidation was tested in reactions with hydrogen peroxide (70 mM, 150 mM and 290 mM) or potassium ferricyanide (25 mM, 50 mM, and 100 mM) in the absence or presence of 1 mM ascorbic acid (AA). The progress of the oxidation was monitored by following the changes in the absorption (Cary50 Bio, Varian) and fluorescence emission spectra (FluoroMax-P, Horiba Jobin Yvon). The spectra were recorded just before addition of an oxidant, and then after being kept in the dark for 5, 15, 30, 45, 60, 75 and 90 min at room temperature. Simultaneously, control (no oxidant) experiments were performed.

RESULTS AND DISCUSSION

The model Crt-less B870 and Crt-containing LH1 complexes were prepared following the published procedures (Fiedor et al., 2004; Fiedor, 2006; Fiedor et al., 2009). The absorption spectrum of B870 exhibits a characteristic maximum at 875 nm assigned to the BChl Qx transition (Fig. 1A). The excitation at the BChl Qx band (590 nm) results in the emission of fluorescence with a maximum at 890 nm. A strong conservative signal, with a crossover point at ~881 nm (Fig. 1B, inset). Other signals appear in the regions of the BChl Qx and Sph absorption bands. The CD spectrum provides a further evidence that the environment of BChl is affected by the presence of Sph.

In order to determine the effect of Crt on the stability of LH1 against chemical oxidants a series of experiments with hydrogen peroxide and potassium ferricyanide was performed. In all cases, a gradual decrease of the LH1 Qx band was observed, accompanied by an increase of absorption at ~690 nm (Fig. 1). The control experiments performed at room temperature in darkness showed that B870 and Sph-LH1 are stable. The decrease of the respective Qx bands did not exceed 7%.

B870 and Sph-LH1 were exposed to 70 mM, 150 mM or 290 mM H2O2. For example, after 5 min treatment of B870 with 70 mM H2O2, the intensity of the BChl Qx band decreased by 10% (Fig. 2A). A prolonged incubation resulted in a further decrease of the absorption band by about 20%, 35% and 63% after 15, 30 and 90 min, respectively. A decrease of the Qx intensity was accompanied by a shift of about 1-2 nm toward longer wavelength in the B870. An increase in H2O2 concentration to 290 mM caused a larger drop of the Qx band, by about 50% and 90% after 15 and 90 min, respectively. A very similar B880 degradation kinetics was observed during the initial 15 min of the treatment of Sph-LH1 with 70 mM H2O2. At longer times, a very clear protective effect of Crt on LH1 is seen, as after 90 min the concentration of Sph-LH1 is twice as high as that of B870 (Fig. 2B). At higher concentrations of H2O2 only a weak effect of Sph was observed (Fig. 2B). The kinetics of emission decrease (not shown) was much faster than the one seen in the absorption but after 90 min a similar 60–70% drop occurred.

The stability of B870 and Sph-LH1 was also monitored in the presence of K3Fe(CN)6. The samples were
exposed to the following concentrations of K3Fe(CN)6: 25 mM, 50 mM and 100 mM. For example, after 5, 30 and 90 min treatment of B870 with 25 mM K3Fe(CN)6, the intensity of the BChl QY band decreased by about 40%, 65%, and 82%, respectively (Fig. 1A, 2C). At the higher concentrations of K3Fe(CN)6, the effect was stronger. The decrease of the absorption intensity was accompanied by a shift of the B870 QY band toward shorter wavelengths (by about 25 nm). No such a shift was observed for Sph-LH1 but the kinetics of the QY shorter wavelengths (by about 25 nm). No such a shift of the absorption spectra of the reconstituted Sph-LH1 complexes was seen in the present work.

Additionally, the effect of ascorbate, a well-known scavenger of ROS (Bodannes et al., 1979), on the kinetics of oxidant-induced degradation of B870 and Sph-LH1 was tested. In the reactions with K3Fe(CN)6, the addition of ascorbate enhanced the stability of all LH1 complexes, howev -

ther act as prooxidants, promoting pigment degradation. The formation of unstable and highly reactive hydroperoxide derivatives of dehydroascorbic acid has indeed been reported (Kwon et al., 1988).

In conclusion, potassium ferri cyanide turned out to be a stronger oxidant than hydrogen peroxide. Both oxidants cause similar changes in the absorption spectra of the complexes, pointing out to the formation of analogous degradation products such as e.g. depsi2-z-acetyl chlorophyll a, as judged from the appearance of a band at ~690 nm in the absorption spectra (Smith et al., 1966). Picorel et al. (1984) or Law & Cogdell (1998) have already demonstrated that oxidation of native LH1 complexes with K3Fe(CN)6 leads to the changes in their spectral properties. Similar changes in the absorption and emission spectra of the reconstituted Sph-LH1 complexes were seen in the present work. The comparison of the degradation kinetics clearly indicates that Crt significantly influences the stability of the complexes, however, it does not prevent them from a gradual irreversible decomposition. The changes in the absorption spectra of LH1 caused by oxidants are slower than the ones in the emission signals. A more rapid drop in the emission intensity can be explained by the fact that the presence of even a small number of excitation traps (oxidized BChl) may completely inhibit the intracomplex energy transfer (Law et al., 1998; Fiedor et al., 2000; Fiedor et al., 2001b).

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