The formation and characterization of the in vitro polymeric aggregates of bacteriochlorophyll c homologs from Chlorobium limicola in aqueous suspension in the presence of monogalactosyl diglyceride

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Abstract

Artificial aggregates of bacteriochlorophyll c (BChl c) were formed in an aqueous medium in the presence of a lipid, monogalactosyl diglyceride (MGDG), and the optical properties of those aggregates were studied by absorption and circular dichroism (CD) mainly. Four BChl c homologs, ([E,E]BChl cF, [P,E]BChl cF, [E,M]BChl cF and [I,E]BChl cF), were isolated from the green photosynthetic bacterium Chlorobium limicola strain 6230. Above 0.0004%, MGDG induced a red-shift of the absorption maxima of BChl c aggregates. At 0.003% MGDG BChl c aggregates showed absorption maxima in the range of 724 to 745 (± 3) nm with a shift of 12 to 24 (± 3) nm depending on the homolog species. Four kinds of BChl c-MGDG aggregates showed characteristic CD spectra. [E,M]BChl cF gave rise to a CD spectrum similar to that of chlorosomes, while the other three gave spectra of opposite sign. These aggregates are sensitive to 1-hexanol treatment; in a saturating amount (0.85%) of 1-hexanol, all the homologs gave a monomer-like absorption spectrum peaking at 670 nm. At an intermediate concentration (0.5%), [E,M]BChl cF showed an enhanced CD intensity, as observed in native chlorosomes. Resonance Raman spectra of the monomer-like BChl c samples indicated that the keto vibrational band at ca. 1640 cm⁻¹ was considerably weakened by the 0.85% 1-hexanol treatment, however the 1680 cm⁻¹ band characteristic of a free keto group did not appear. These results indicate that the artificial aggregates formed by purified BChl c homologs and MGDG are good models for studying chlorosomes structure.

Abbreviations: BChl – bacteriochlorophyll; CD – circular dichroism; MGDG – monogalactosyl diglyceride; [E,E]BChl cF – 8-ethyl-12-ethyl farnesyl BChl c ; [P,E]BChl cF – 8-propyl-12-ethyl farnesyl BChl c ; [E,M]BChl cF – 8-ethyl-12-methyl farnesyl BChl c ; [I,E]BChl cF – 8-isobutyl-12-ethyl farnesyl BChl c (This nomenclature is explained by Smith (1994))

Introduction

Chlorosomes are the main light-harvesting bodies in green photosynthetic bacteria (Olson 1980). Each chlorosome contains about 10,000 BChl c molecules in self-aggregated forms (Krasnovsky and Bystrova 1980), and at least three kinds of polypeptides are known in chlorosome preparations (Feick and Fuller 1984). These polypeptides are postulated to be present in the envelope made of a monolayer of monogalactosyl diglyceride (MGDG) (Olson 1980; Blankenship et al. 1988). All the proteins can be extracted from
chlorosomes without changing their spectral properties (Holzwarth et al. 1990), and a chlorosome-like structure can be formed in aqueous suspension using protein-free lipid-pigment extracts from chlorosomes (Hirota et al. 1992a). This strongly suggests that in vivo BChl c aggregates might be formed without interaction with proteins (Worcester et al. 1986; Holzwarth et al. 1990; Hirota et al. 1992a).

In the case of *Chlorobium limicola* strain 6230, four homologs are known: [E,E]BChl cF, [P,E]BChl cF, [E,M]BChl cF and [I,E]BChl cF (Olson and Pedersen 1990; Smith et al. 1982). The first two are major constituents and the last two, minor ones. To understand the molecular organization of BChl c aggregates in chlorosomes, reconstitution was carried out in non-polar solvents (Olson and Pedersen 1990; Olson and Cox 1991; Uehara and Olson 1992; Causgrove et al. 1993) and in aqueous systems using lipidic components including BChl a, carotenoids, and lipid (Hirota et al. 1992b; Miller et al. 1993). For a further detailed analysis, optical properties of individual homologs and their contribution to the total properties are necessary.

In this study we reconstituted BChl c aggregates consisting of only one BChl c homolog in the presence of MGDG, in which the essential constitution of native chlorosome was expected to be reproduced. Because MGDG also occurs in the chlorosome, this study might represent another step toward reconstituting the chlorosome from defined component parts. Optical properties were monitored by absorption, CD and Raman spectroscopy. In some cases the reconstituted aggregates showed optical properties similar to those of chlorosomes. Based on the molecular structure of the homologs, the molecular organization in chlorosomes is discussed.

**Materials and methods**

[E,E]BChl cF, [P,E]BChl cF, [E,M]BChl cF and [I,E]BChl cF (Table 1) were isolated from *Chlorobium limicola* strain 6230 (formerly called 'Cb. limicola f. thiosulfatophilum') and purified according to the method described by Olson and Pedersen (1990). No contamination by BChl a or carotenoid was found. Monogalactosyl diglyceride (MGDG) from whole wheat flour was furnished by Sigma chemical company, and a GR-grade reagent of 1-hexanol (Aldrich or Wako Pure Chemical Industries) was used without further purification.

For the preparation of aggregates the isolated BChl c homolog(s) and MGDG were dissolved in methanol and injected into vigorously stirred phosphate buffer (1 mM, pH 7.01) cooled by ice-water (final methanol concentration 0.5 vol%). When necessary, microliter aliquots of 1-hexanol were injected into the aggregate solution and shaken to dissolve. All operations were performed under dim light. MGDG concentration was kept to 0.003% in this study, unless otherwise specified.