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Estimation of the bacteriochlorophyll *c* oligomerisation extent in *Chloroflexus aurantiacus* chlorosomes by very low-frequency vibrations of the pigment molecules: A new approach

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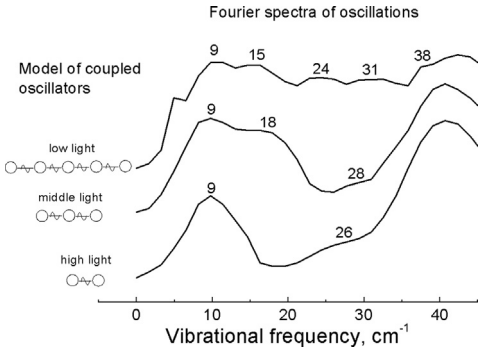
HIGHLIGHTS

- Very low-frequency vibrations in *Cfx. aurantiacus* chlorosomes are sensitive to oligomerisation.
- Theory of coupled oscillators predicts the very low-frequency BChl *c* vibrations.
- The very low-frequency BChl *c* vibrations depends on growth-light-intensity.
- The unit building blocks are built up from quasi-linear chains of several BChl *c* pigments.

GRAPHICAL ABSTRACT



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ABSTRACT

In green photosynthetic bacteria, the chlorosomal bacteriochlorophyll molecules are organized via self-assembly and do not require proteins to provide a scaffold for efficient light harvesting. Despite numerous investigations, a consensus regarding the spatial structure of chlorosomal antennae has not yet been reached. For the first time, we demonstrated by coherent femtosecond spectroscopy at cryogenic temperature that the very low-frequency ($\sim 10^1$ cm^{-1}) vibrations of bacteriochlorophyll *c* pigments in isolated *Chloroflexus aurantiacus* chlorosomes are sensitive to their oligomerisation extent which depends on the light intensity during the growth of the cell cultures. We explained this sensitivity in terms of the coupling of delocalised vibration modes of BChl *c* molecules aggregated into chains within their antenna unit building blocks. These findings, together with previously obtained spectroscopy and microscopy data, confirmed that the unit building blocks functioning within *Chloroflexus aurantiacus* chlorosomal antenna are built up from the rather short (2–5 BChl *c* pigments) quasi-linear chains. The approach presented here seems to be perspective since it directly reveals structural and dynamical properties of the oligomeric systems.

Abbreviations: Δ*A*, light − dark absorbance changes; BChl, bacteriochlorophyll; *Cb*, *Chlorobaculum*; *Cfx*, *Chloroflexus*; Chl, chlorophyll; *C*, *Chlorobium*; CMC, chlorosome-membrane complexes; FWHM, full width at half maximum; OD, optical density; RC, reaction centre; *Rba*, *Rhodobacter*

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1. Introduction

Photosynthesis is the key natural process which underlies of life on Earth. Solar energy is converted into the energy of stable chemical compounds in this biological process. Photosynthesis is the main source of oxygen and organic compounds on Earth. Solar energy refers to an inexhaustible and ecologically pure form of energy.

Photosynthesis starts from absorption of light quanta in light-harvesting chlorophyll complexes. Then the excitation energy is transferred to the photosynthetic reaction centres, where primary conversion of the light energy into the energy of separated charges occurs. These primary events trigger a long and very complicated series of biochemical reactions resulted in a synthesis of stable chemical compounds.

The quantum efficiency of primary steps of natural photosynthesis is close to 100% [1] due to the rigid optimization of the photosynthetic apparatus structure according to the functional criteria [2,3]. The requirements for structural optimization become more stringent with increasing the antenna size [4]. Therefore the mechanisms of primary processes and the features of the photosynthetic apparatus that ensure its high efficiency for light energy conversion must be explored. This subject has compelling importance for both natural and artificial photosynthesis.

Previously, it was shown by the model calculations, that antenna pigment oligomerization is biologically expedient strategy for light harvesting in photosynthesis [5,6]. Oligomerization of antenna pigments is possible due to intrinsic donor-acceptor properties of all chlorophylls. These key properties of chlorophylls make possible self-aggregation of the pigments [7,8].

The most amazing example of long-range ordered natural light harvesting antennae are the chlorosomes of green photosynthetic bacteria. Chlorosomes are the largest among all known photosynthetic light-harvesting structures ($\sim 10^4$–10^5 pigments in the aggregated state). A chlorosome body of ellipsoidal shape (100–200 × 40–100 × 10–30 nm) is attached to the inner surface of the cytoplasmic membrane. In the green nonsulphur thermophilic bacterium, *Chloroflexus* (*Cfx*.) *aurantiacus*, the chlorosome is the peripheral antenna that is typically comprised of approximately 10^4 bacteriochlorophyll (BChl) *c* pigments [9]. Direct experimental proof of oligomeric organization of chlorosomal pigments in intact cells of the green bacteria *Cfx*. *aurantiacus, Chlorobium* (*C*.) *limicola and Chlorobaculum* (*Cb*.) *pheovibrioides* was obtained by the spectral hole-burning method [10–12]; and the chlorosomal aggregate has been shown to be built from quasi-linear chains of BChl *c* molecules. A long-range molecular order of chlorosomal BChl *c* of the green bacteria *Cfx*. *aurantiacus* and *C. limicola* was shown by picosecond fluorescence polarisation spectroscopy [13,14].

Besides BChl *c/d/e*, chlorosomes of all green bacteria also contain carotenoids and a small amount of BChl *a* [15]. This BChl *a* antenna connects the chlorosomal BChl *c* antenna with the cytoplasmic membrane, in which the core BChl *a* antenna and reaction centres are located [9,15]. The harvested energy is transferred from BChl *c* aggregates to the BChl *a* baseplate within 10–40 ps, depending on the size of the BChl *c* antenna [16]. As with most photosynthetic organisms, these bacteria are able to adapt to low light intensities by drastically increasing their peripheral antenna size [17–21, this work].

The most interesting and attractive feature of the chlorosome is the fact that BChl molecules self-aggregate into ordered structures without participation of proteins as a structural matrix. It has been widely accepted that the neighbouring molecules in the chlorosomal BChl aggregates couple with each other via coordination bonds between the $C3^1$ hydroxy group and the central Mg atom and via hydrogen bonds between the C13 carbonyl group and the $C3^1$ hydroxy group [7,8].

Structural models of the chlorosomal BChl aggregates have been extensively investigated [14,16,22–27]. Some models used a rod-like arrangement of BChl *c* aggregates based on freeze-fracture electron microscopy data [16,22,23]. Conversely, some models used a lamellar-type arrangement of the BChl aggregates in chlorosomes based on X-ray structure analysis [24]. Concentric helical nanotubes of pairs of alternating *syn*-antiligated BChl *c* and *d* stacks were proposed for the green sulphur bacterium, *Cb. tepidum*, mutant [25]. Besides, a concentric-roll structure of antennae was proposed in [26,27]. The variety of structural models can be a consequence of structural heterogeneity of the chlorosomes. Availability of the various types of molecules (BChl *c, d, e*) and large variations in the size of their aggregates strongly complicated the use of X-ray structure analysis. This inherent heterogeneity affects the use of various optical spectroscopy methods due to ensemble averaging of important spectral features. The single-particle spectroscopy of individual chlorosomes also confirmed the strong structural disorder of them. A combination of several techniques, such as mutagenesis, single-particle spectroscopy, and cryo-EM imaging is useful for study the structural arrangement of the aggregated BChl molecules inside the chlorosome [25]. Despite numerous investigations, a consensus regarding the 3D structure of the chlorosomal antennae has not yet been reached (for review, see [21] and the references therein).

Previously, the growth-light-controlled variability of the aggregation extent of peripheral antenna pigments was studied in isolated *Cfx. aurantiacus* chlorosomes by steady-state and time resolved spectroscopy of the BChl *c* Q_y absorption bands [16,19–21]. The experimental findings were theoretically explained in terms of unit building blocks from which the rod-like structures of BChl *c* aggregates are composed in *Cfx. aurantiacus* chlorosomes. Each rod was considered as a linear chain of the unit building blocks in accordance with the freeze-fracture electron microscopy data [16,22,23]. In its turn, each unit building block was composed of several short BChl *c* chains. The size of the BChl *c* unit building block as well as the number of BChl *c* pigments in quasi-linear chains within the unit building block were estimated using the standard exciton theory. The tubular model of 6 exciton-coupled BChl *c* chains within the unit building block and inter-chain distances of ~2 nm ensured the best fit of the experiment and approximated the in vivo BChl *c* low packing density [16,19–21]. The number of BChl *c* molecules per each chain within the unit building block was found to be not > 6–7 [21]. This model was able to explain several key spectroscopic properties of *Cfx. aurantiacus* chlorosomes, such as the exciton level structure of BChl *c* aggregates, revealed by spectral hole burning experiments, the antenna-size-dependent exciton dynamics in the BChl *c* chlorosomal antenna, the temperature dependence of the steady state fluorescence line shape of the BChl *c* band and the femtosecond pump-probe spectra of the BChl *c* chlorosomal antenna.

In the present work, we suggest a new approach to estimation of the BChl *c* oligomerisation extent in *Cfx. aurantiacus* chlorosomes by study of low-frequency vibrations of the aggregates. A set of intermolecular vibrational frequencies provides a deep insight into the structure of the molecular aggregate. There are at least three essential problems in this approach. First, it is clear that the true intermolecular vibrations of a large molecular assembly have very low frequencies. Two widely used techniques such as stimulated Raman and coherent pump-probe spectroscopy are not able to reveal modes with frequencies lower than several cm^{-1}. Second, a real motion of large molecular aggregates is an extremely complicated mixture of inter- and intramolecular motions, and it is not clear how to separate them. Third, the known low-frequency BChl *c* modes of *Cfx. aurantiacus* chlorosomes are not assigned. Nevertheless, an approach presented here seems to be perspective because it directly reveals structural and dynamical properties of the nuclear subsystem.

The present study compared the coherent components of the kinetics measured in the BChl *c* Q_y bands of *Cfx. aurantiacus* chlorosomes, which were isolated from cells grown under different light intensities and therefore had different sizes of BChl *c* antennae and their unit building blocks [19–21]. Pronounced coherent low-frequency (50–250 cm^{-1}) oscillations were previously found in *Cfx. aurantiacus* chlorosomes at room [28] and cryogenic [29] temperatures. The weaker low-frequency oscillations were found in chlorosomes isolated