X-ray scattering study on the effect of carotenoid biosynthesis to chlorosome structure

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Chlorosomes are light harvesting complexes from photosynthetic filamentous and green sulfur bacteria that are able to survive in low-light conditions. A typical chlorosome is an ellipsoidal body (typically 100-200 nm long, 20-50 nm in diameter) that is composed mainly of bacteriochlorophylls and carotenoids with minor contribution from quinones, lipids, and proteins.

The genome of Chlorobium tepidum, a mesophilic, photoautotrophic green sulfur bacterium which contains chlorosomes was sequenced in 2002 [1]. Genetic manipulation and engineering of knockout-strains of this bacterium has recently become possible [2]. Also, a lamellar model of bacteriochlorophyll (BChl) and carotenoid organization in chlorosomes was proposed based on X-ray scattering and electron microscopy data from wild type Chlorobium tepidum chlorosomes [3], contradicting the cylindrical model of BChl organization favored previously in the literature.

The proposed lamellar model led to the question of the role of carotenoids in the chlorosome structure. The working hypothesis was that changes in the types of carotenoids and their concentration inside the chlorosome would lead to a modulation in the parameters of the lamellar model, especially to the distance between lamellas, which in the wild type chlorosome is 21 Å, corresponding to a maximum in X-ray scattering at \( q = 0.30 \text{ Å}^{-1} \).

The carotenoid biosynthesis requires many steps and enzymes. The enzymes disabled in this experiment and the genes corresponding to them are, in the order of their occurring in the carotenoid synthesis pathway, phytoene synthase (crtB), phytoene desaturase (crtP), \( \zeta \)-carotene desaturase (crtQ) and carotenoid cis-trans isomerase (crtH), which together produce \( \gamma \)-carotene starting from geranylgeranyl diphosphatate [2]. From there on the carotenoid biosynthesis branches, producing a variety of carotene and chlorobactene end products. Two enzymes affecting this stage of synthesis, \( \gamma \)-carotene desaturase (crtU) and carotenoid 1’,2’-hydratase (crtC) were also disabled in this study.

Chlorosomes from several mutant strains of Chlorobium tepidum, each lacking one of the aforementioned genes were isolated and concentrated to thick solutions. X-ray scattering from the solutions was measured at the Hasylab beamline A2. The wavelength of the X-rays was 1.5 Å and the distance from the sample to the CCD detector was 435 mm. The samples were held in 1 mm thick cuvettes with a silver frame and two 13 \( \mu \)m Kapton windows.

The X-ray scattering intensities, presented in figure 1, show that disabling the genes affecting the late stages of differentiating carotenoid biosynthesis (crtH, crtU, crtC), has only a small effect on the scattering. The peak positions and widths vary, but the overall shape of the scattering curve remains similar to the wild type.

Disabling the genes on the early part of the carotenoid biosynthesis has a more pronounced effect. For the mutants lacking crtQ and crtB genes, the large angle part of the scattering remains similar to the wild type, but a broad maximum at around \( q = 0.1 \text{ Å}^{-1} \) suggests that carotenoid
Figure 1: X-ray scattering intensities of chlorosomes from knockout mutants of *Chlorobium tepidum* compared to the wild type. The curves can be divided into two groups where the scattering is qualitatively similar (left panel) to the wild type, or differs from it (right panel). The grouping corresponds well to carotenoid biosynthesis. The genes disabled in the samples on the right panel are used in the early stages of the synthesis producing chlorosomes deficient in all types of carotenoids. In the samples in the left panel, the disabled genes only affect the later stage of synthesis, producing chlorosomes lacking in only some types of carotenoids, which has a smaller effect on the structure.

deficiency either modifies the X-ray scattering contrast of the chlorosome, causing a hitherto unknown form factor of the BChl aggregates inside the chlorosome to become visible, or that the lack of carotenoids changes the internal structure of the aggregates themselves. In either case, the chlorosome model needs qualitative changes.

The X-ray scattering intensity from the crtP lacking mutants differs even more from the wild type and shows diminished order in the BChl aggregates. Since the mutants lacking the preceding enzyme coded by crtB in the reaction pathway show less change from the wild type, it remains to be seen if the effect observed is real, or caused by a defective sample.

References