The enzyme responsible for the methylation at the C-20 methine position of the bacteriochlorophylls c and e found in green sulfur photosynthetic bacteria has been identified by genomics and knockout mutagenesis. The distribution of this enzyme in other green sulfur bacteria is surprising.

ULTRaweak Light Collection by Green Photosynthetic Bacterial Antennas

The green photosynthetic bacteria are the world champions of doing photosynthesis at low light intensities (7). They accomplish this by using a unique antenna complex known as a chlorosome, which is packed with specialized photopigments, bacteriochlorophylls c, d, and e (1). These unusual chlorophylls are not found in any other organisms, and they self-assemble into large pigment oligomers with almost no involvement of protein. This biosynthetically “cheap” antenna complex has an enormous absorption cross-section and permits certain of these organisms to live at the lowest light intensities known to support photosynthesis, up to a million times lower light than normal sunlight (8). At these low intensities, each bacteriochlorophyll absorbs a photon approximately once every 8 h! A key step in the biosynthesis of bacteriochlorophylls c and e (but not d) is the methylation at the C-20 position of the chlorin macrocycle (Fig. 1). The enzyme that carries out this methylation (BchU) has previously not been identified. However, in a recently published paper, Maresca et al. (4) identify the methyltransferase enzyme in Chlorobium tepidum by using a combination of comparative genomics and knockout mutagenesis.

The investigators did some clever detective work to identify the gene that codes for the C-20 methyltransferase. The complete Chlorobium tepidum genome sequence has been determined (3), but the genome contains many potential methyltransferase genes and initial guesses were not correct. The identification was made by comparative genomics with the filamentous anoxygenic phototrophic (FAP) bacteria, which also contain the green nonsulfur bacteria. These organisms are the only other major group of bacteria that contain the chlorosome antenna complex, although overall they are not close relatives of the green sulfur bacteria, and a draft genome is available for one member of this group, Chloroflexus aurantiacu.s. Fortunately, genes that code for chlorosome components are clustered in the FAP bacteria, while they are not in the green sulfur bacteria, and this clustering suggested a methyltransferase gene as a good candidate for the C-20 methylase. A knockout mutation in Chlorobium tepidum had the expected phenotype of containing bacteriochlorophyll d, which lacks the C-20 methyl group, instead of bacteriochlorophyll c. This established the identity of the gene, which was named bchU (4).

A Surprise Finding and Some Predictions

Other groups of green photosynthetic bacteria contain functional chlorosomes that contain only bacteriochlorophyll d. Maresca et al. (4) examined some of these strains. A surprise in their findings is that at least one “wild-type” bacteriochlorophyll d-containing organism also contains a bchU gene, but with a frameshift mutation that leads to premature termination and an inactive enzyme. Certain bacteriochlorophyll d-containing strains have long been known to be prone to reversion to making bacteriochlorophyll c when grown for extended periods at low light intensities (2). In some of these strains, Maresca et al. (4) found that a second mutation restores the original reading frame and results in an active methyltransferase enzyme. Interesting questions remain about the observed distribution of these bacteriochlorophyll d-containing organisms in nature and how the C-20 methyl group affects the efficiency of light collection. In laboratory growth competition experiments, Maresca et al. (4) found that the bacteriochlorophyll d-containing Chlorobium tepidum bchU mutant cells did not grow as rapidly as the bacteriochlorophyll c-containing wild type under low light conditions but grew at the same rate at higher light intensity.

Can it be that all bacteriochlorophyll d-containing strains (which are often dominant isolates at somewhat higher positions in the water column and therefore higher light intensities) really contain a bchU gene that is inactivated by a frameshift and susceptible to reversion? This seems unlikely, but this question could be easily resolved by analysis of a series of bacteriochlorophyll d-containing strains. It may be that some of the common laboratory bacteriochlorophyll d-containing strains have arisen in the laboratory by selective pressure due to culturing at higher light intensities and that newly isolated bacteriochlorophyll d-containing strains will lack the gene entirely.

There is one additional pigment in the series of pigments
chlorophylls e absorb at about 690 to 710 nm. It is a bit of a mystery why, in that bacteriochlorophyll C-7. According to the progression of in vivo absorption maxima, niche that is not well exploited, as it is just to the red of the predicted 690- to 710-nm spectral window would appear to be found, as both of the enzymes that make the two functional groups that are not widely distributed.

It should be possible to produce an organism that contains bacteriochlorophyll f, simply by knocking out the C-20 methytransferase enzyme in a bacteriochlorophyll e-containing strain. While none of the bacteriochlorophyll e-containing strains have genetic systems yet available, this should still be relatively straightforward.

A final puzzle yet to be solved is the identification of the enzyme that makes the formyl group at the C-7 position in bacteriochlorophyll e. This is the same position and functional group that is found in chlorophyll b. However, the enzymes are almost certainly not homologous, as the enzyme that makes chlorophyll b is a mixed-function oxidase that relies on O2 as a substrate (9) and the bacteriochlorophyll e-containing bacteria are strict anaerobes. This is almost certainly another in a growing group of cases of gene replacement, in which the same biosynthetic steps are carried out by entirely different enzymes in anaerobic and aerobic organisms, with only the aerobic enzymes using O2 as a substrate. Other examples include the coproporphyrinogen oxidase involved in heme and chlorophyll biosynthesis (HemN versus HemF), the oxidative cyclase that makes the isocyclic ring in chlorophylls (BchE versus AcsF), and ribonucleotide reductase (NrdG versus NrdB) (6). The anaerobic versions are probably the more ancient enzymes, dating to a time more than 2.2 billion years ago when the earth was largely anaerobic, and the more efficient aerobic versions that use the powerful oxidant O2 have replaced the older ones whenever possible.

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