Volume 52(2):333-356, 2008
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Macro-organization and structural flexibility of the light-harvesting system of diatoms (Bacillariophyceae) and their significance in the photosynthetic light energy utilisation

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Among the phytoplankton species of marine and freshwater communities, diatoms play a dominant role in the biogeochemical cycles of carbon, nitrogen, phosphorus and silicon with a strong impact on the global climate. Since diatoms experience randomly fluctuating light intensities and large scale temperature changes, they have developed various mechanisms of photoprotection.

In higher plants, it has been established that the photosystems (PSs) with their peripheral chlorophyll *a/b* light-harvesting antenna complexes (LHCs) form supercomplexes. The PSII-LHCII and PSI-LHCI supercomplexes are laterally segregated in the granal and stromal thylakoid membranes, respectively (cf. e.g. Mustárdy and Garab 2003). It has also been shown that LHCII and PSII-LHCII are assembled into macrodomains with long-range chiral order, which possess remarkable structural flexibility and by this means the structural flexibility of the macroassemblies plays an important role in the regulation of the light energy conversion (Garab 1996).

Diatoms contain specialized peripheral light-harvesting antennas, the fucoxanthin-chlorophyll *a/c* proteins (FCPs), instead of LHCs. FCPs are also intrinsic light-harvesting complexes but their carotenoid is the fucoxanthin and contains chlorophyll *c* as accessory pigment. Compared to higher plants, our knowledge concerning the arrangement and the supramolecular organization of the antenna complexes in the thylakoid membranes is quite rudimentary, and much less is known about their possible role in different regulatory processes.

The major aim of our studies was to characterize the (macro-)organization of the complexes in *Phaeodactylum tricornutum* and *Cyclotella meneghiniana* cells, as well as on isolated thylakoid membranes and FCPs. By using circular dichroism (CD) spectroscopy, we found that the spectra of the whole cells were dominated by an intense band at (+)698 nm, with typical psi-type features (psi, polymerization or salt-induced). This band, which appeared to be associated with the multilamellar membrane architecture, was sensitive to the light intensity during growth, to the osmotic pressure of the medium and to heat. We also found that it was capable of undergoing reversible changes upon illumination with actinic light. In isolated thylakoid membranes, the psi-type CD band, which was lost during the isolation procedure, could be partially restored by addition of Mg^{2+} ions; the same treatment was also important for optimizing the quantum yield of PSII and the non-photochemical quenching of chlorophyll *a* (Szabó et al. 2008). With a refined isolation method, we were able to isolate the oligomeric form of FCP, which represented the native form of the antenna system in thylakoid membranes of diatoms (Lepetit et al. 2007). We also gained information on the orientation and local environment of a special fucoxanthin pigment molecule of the FCP, which exhibited an extremely strong electrochromic response and intense linear dichroism (LD) signal at around 550 nm, most probably given rise by strong fucoxanthin/chlorophyll *c* interaction.

In summary, our data have shown the presence of highly flexible macroassemblies of the light-harvesting system in diatoms, which also appears to participate in different regulatory processes of the photosynthetic light energy conversion.

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Characterization of beta-glucosidase enzymes and their coding genes from the fungal class Zygomycetes

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The genus *Rhizomucor* (Zygomycetes, Mucorales) comprises two well-established thermophilic species, *R. pusillus* and *R. miehei* (Vágvölgyi et al. 1999). Both of them are well known from biotechnological applications in consequence of their effective extracellular enzymes, e.g. proteases and lipases (Rao et al. 1998). Beta-glucosidases play important roles in biology, including the degradation of cellulose biomass by fungi and bacteria, degradation of glycolipids in mammalian lysosomes, and the cleavage of glycosylated flavonoids in plants (Bhatia et al. 2002).

Filamentous fungi are known to be good producers of beta-glucosidases and several fungal glucosidases have been isolated and analyzed. Unfortunately, Zygomycetes are poorly characterized from this aspect. In the frame of a recent study, beta-glucosidase activity of

several Zygomycetes fungi was tested in solid-state fermentation assays. Some *R. miehei* strains showed intensive extracellular enzyme activity. The aim of our present study is the identification and molecular and biochemical characterization of a beta-glucosidase enzyme and its coding gene (*bgl*) from *R. miehei*.

Degenerated beta-glucosidase-specific primer pairs were designed to conserved regions of fungal glycoside hydrolase family 3 genes and a 493 bps long fragment was amplified by PCR from the genomic DNA of the *R. miehei*. The sequence of the amplicon was determined; it showed high homology with the C-terminal domains of the beta-glucosidases belonging to the family 3. Based on this sequence, specific primers were designed for inverse PCR. The original fragment has been lengthened to a 4063 bps long sequence which contained the 2826 bps long beta-glucosidase gene encoding a protein with a length of 743 amino acids. *Rhizomucor bgl* showed the highest homology with the beta-glucosidases of *Phanerochaete chrysosporium*, *Trichoderma reesei* and *Piromyces* sp. strain E2.

For gene expression studies two transformation vectors were constructed: the plasmid pTM1 contained *bgl* under the control of the regulator sequences of the related *Mucor circinelloides gpd1* gene, while the plasmid pTM4 harboured the promoter region of the *bgl* fused with a green fluorescent protein gene. In the lack of an efficient transformation system in *R. miehei*, genetic transformations were started in a heterologous system: PEG-mediated protoplast transformations were performed in an uracile auxotrophic *M. circinelloides* strain. Induction of the *bgl* promoter by different substrates was studied in the *M. circinelloides* transformants harbouring the pTM4 plasmid. Strong fluorescence was observed only in the transformants growing on cellobiose containing medium. Analysis of the transformants containing pTM1 is in progress.

For production of the extracellular beta-glucosidase enzyme in high amount, *R. miehei* was grown on wheat bran medium for six days at 40°C. The enzyme was purified from the crude extract to homogeneity by ammonium sulphate fractionation and two-step chromatographic separation through Sephadex G100 and G200 columns was performed. The molecular mass of the purified enzyme was determined by SDS-polyacrylamide gel electrophoresis. The optimum temperature and pH for the action of the enzyme were at 60°C and 4.0 to 5.0, respectively; the beta-glucosidase proved to be highly stable at temperatures up to 50°C but it almost lost its activity at temperatures above 70°C. The enzyme was fairly stable at pH 4.0 to 6.0 and 20% of the activity remained after incubation at pH 3.0.

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Map based cloning of leaf developmental abnormality in *Medicago sativa* and comparison of the rDNA (NOR) regions in *Medicago truncatula* and *Medicago sativa*

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A *Medicago sativa* mutation called sticky leaf (*stl*), which appears in the nature occasionally was reported previously (Stanford 1959) and used as a morphological marker in cross-fertilizations. The *stl* mutant is characterized by the adhesion of the adaxial sides of adjacent leaflets in the same leaf, as well as adhesion of opposite halves of the same leaflet. The inheritance of the mutant in tetraploid populations suggested that the *stl* character is determined by a single recessive gene (Stanford 1965). This was confirmed later in a diploid F2 segregating population originating from a cross between *M. sativa* ssp. *quasifalcata* and *M. sativa* ssp. *coerulea* (Endre G. PhD thesis 1997). Genetic mapping of this trait in this population placed the *Stl* gene on Linkage Group six (LG6) in the close vicinity of the rRNA coding region (NOR). In the closely related model legume *M. truncatula* that shows very high overall macrosynteny with *M. sativa* the position of NOR region is different, it is located on LG5.

Our aim is to identify the mutant gene which is responsible for the *stl* phenotype in *M. sativa* with the help of the available genomic information of the model *M. truncatula*. For this the localization of the ortholog *Stl* gene in *M. truncatula* is needed, therefore we compare the NOR and its flanking regions in these *Medicago* species. To explore this syntenic relationship we used three approaches. One of these was to map the molecular markers linked to the *M. sativa stl* trait on the *M. truncatula* linkage map. The second direction was to search for and use structural genomic information of *M. truncatula* sequenced BAC clones carrying rDNA sequences. We have identified the repeat unit sequences and looked for discrete sequences for mapping on *M. sativa*. As a third approach we have screened a *M. truncatula Tnt1* insertional mutant plant collection for leaf phenotypes similar to *stl*.

Molecular markers Q5C and P16C closely linked to *stl* phenotype on the *M. sativa* map were used to identify *M. truncatula* BAC clones. Some have already been mapped in *M. truncatula*, but their position was neither on LG5 near NOR region nor on LG6 in syntenic position of *stl* in *M. sativa*. We have subcloned and sequenced other BACs with unknown location but only intergenic repetitive sequences were identified not suitable for syntenic mapping purposes.

Following the second approach with the help of BAC sequences we determined the rDNA units (the 18S-5.8S-25S rRNA coding + IGS sequences) in *M. truncatula*. The thorough analyses of these BACs identified only one putative other coding sequence but so far mapping efforts failed in *M. sativa*.