indicates that electrostatic interactions are important for the protein pre-orientation. The following gradual rearrangement increases the overlap of nonpolar surface areas leading to an electron transfer active complex. In order to characterize the influence of different interaction contributions in detail, we studied a cross complex of Nostoc cyt f and Phormidium pc [3]. Our results indicated that this complex interacts with an affinity that is intermediate between those of the Nostoc complex and Phormidium complex. The lower net charge of pc in Phormidium decreases but not abolishes the attraction to cyt f, resulting in the formation of an encounter complex that is more diffuse than that of the Nostoc complex. The most affected amino acids of pc are located at its hydrophobic patch, indicating a direct interaction of this patch with the active site of cyt f. Thus, electrostatic interactions direct pc towards the active center of cyt f, but the final complex is predominantly stabilized hydrophobically.

References

S11.P5

Systems bioenergetics of chloroplast revisited towards ecotoxicity assessment: Fluorescence dynamics of a substituted aminoacridine to study the effects of one EU-approved and three EU-banned photosynthetic inhibiting herbicides on thylakoid membranes of the weed Chenopodium album
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It is well established that the energization of chloroplast membranes induces a significant increase of negative surface charge density, as demonstrated from measurements of the electrokinetic potential and the adsorption of cationic probes [1, 2]. In the present study, the potential toxicity to the bioenergetic functions of isolated common lambsquarter (Chenopodium album) chloroplasts of four commercial herbicide formulations, one EU-approved (linuron) and three EU-banned (atrazine, simazine and paraquat), was tested. Specifically, this study aims to validate the use of the cationic probe 9-amino-6-chloro-2-methoxyacridine (ACMA) fluorescence quenching method in weed chloroplasts and applies this method to assess the capacity of photon-induced membrane potential generation after individual herbicide exposure. After chloroplast isolation technique optimization, the kinetics of thylakoid membrane energization were examined with ACMA when the photosynthetic inhibitors were added in vitro. The kinetics of the probe were identical for linuron, atrazine and simazine. The concentration-dependent effects of simazine were approximately 10 times higher (0–50 μM) than the other two photosystem II inhibitors (0–4 μM). The ACMA kinetic curves were different for paraquat: similar to simazine, this photosystem I inhibitor exerted concentration-dependent phytotoxic effects 10 times higher (0–50 μM) than atrazine and linuron. The results demonstrated the usefulness of the ACMA as a simple bioassay tool that may find further use in research of both chloroplast bioenergetics of weeds and environmental toxicology.

References

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S11.P6

We're stronger together — The tale of LHClI and liposomes
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The photosynthetic membrane of a chloroplast consists of lipids (both polar and non-polar ones), photosynthetic reaction centres, electron transporters and many others [1]. It is probably the most complex of all membranes with respect to both structure and function. The chloroplast membrane must conduct many biochemical reactions that have to be regulated in response to different temperatures and light condition changes [2]. It has to cope with destructive effects of both light and oxygen stress, and repair itself constantly if necessary [2]. Interoperation of all membrane’s elements is vital for its proper functioning. We tried to focus only on two components: the light harvesting pigment–protein antenna complex of photosystem II (LHClI) isolated from spinach thylakoids and plant galactolipids such as monodigalactosyldiacylglycerol (MGDG), digalactosyldiglycerol (DGDG) and phosphatidylglycerol (PG). Isolated LHClI is often used as a model system to study the photosynthetic apparatus under different conditions [3]. The aim of this work is to determine mechanisms and types of interactions between LHClI and its lipid surrounding. To achieve this goal we used several spectroscopic methods like circular dichroism, infrared spectroscopy, low-temperature fluorescence and fluorescence lifetime measurements. The changes in protein aggregation were studied. Spectroscopic data showed the type of protein–protein and lipid–protein interactions during membrane stacking. Examination of the type of interactions observed in an artificial, less complicated system, makes the organization mechanisms of specific thylakoid membrane in vivo foreseeable. Acknowledgments KG acknowledges the National Science Centre, Poland for financial support – FUGA grant no 2013/08/S/NZ1/00823.

References

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S11.P7

Production of active heterodimeric cytochrome bc1 enzymes
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The ubihydroquinone: cytochrome c oxidoreductase, or cytochrome bc1 is a central component of photosynthetic and respiratory energy transduction pathways in many organisms. It contributes to the generation of membrane potential and proton gradient used for ATP production. This enzyme is a structural dimer formed of two intertwined monomers, with each monomer consisting of the Fe/S protein, cytochrome b and cytochrome c1 subunits. Its unusual three-dimensional architecture raises the questions of whether the monomers operate independently, or they cooperate during the catalytic cycle of the enzyme. Here, using the facultative phototrophic bacterium Rhodobacter capsulatus, we developed a new genetic approach that allows the study of intra and inter-monomer interactions within the cytochrome bc1. The approach consists of two-plasmids carrying two independent petABC operons encoding cytochrome bc1. This “two-plasmids” system is genetically stable especially in a RecA-deficient background, produces both homo- and hetero-dimeric mutant variants of the enzyme, and supports normal photosynthetic growth of R. capsulatus. Both inactive homodimeric and active heterodimeric cytochrome bc1 variants were purified to homogeneity from the same cells, and purified samples subjected to mass spectrometry analyses. These data along with the EPR characteristics showed unequivocally that the cytochrome b subunits carried the expected mutations and their associated epitope tags. Interestingly, kinetic data showed equilibration of electrons between the four b heme cofactors of the heterodimer; via reverse inter-monomer electron transfer, providing further support for the heterodimeric Q-cycle model of cytochrome bc1 mechanism of function. (This work is supported by NIH GM38237 and Division of Chemical Sciences, Geosciences and Biosciences, Office of Basic Energy Sciences, DOE DE-FG02-91ER20052.)

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S11.P8

Integration of energy and electron transfer reactions in plant thylakoids
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The photosystem II (PSII) complex uses solar energy for photosynthetic electron transfer to the cytochrome b6f (cytb6f) complex, which conserves energy as an electrochemical proton gradient. Electron transfer between the two complexes involves exchange of the lipophilic electron carrier plastoquinone. Two competing models for the organization of cytb6f complexes with respect to PSII complexes exist, which imply short or long range diffusion of plastoquinones between them. Here, we describe the results of our structural investigation into the organization of cytb6f with respect to PSII in the plant photosynthetic membrane using atomic force microscopy. The findings reveal how plants optimize photosynthetic efficiency by balancing the competing requirements of solar energy harvesting and electron transfer.

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