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


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S11.P4

On the origin of ferredoxin-induced fluorescence changes in thylakoids

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Addition of NADPH and ferredoxin (Fd) to thylakoid preparations in the dark results in a characteristic slow increase in chlorophyll fluorescence with a half-time measured in tens of seconds which plateaus at approximately 15% of the fluorescence observed during a saturating actinic flash. This phenomenology, which we call $F_{\text{rise}}$, is sensitive to antimycin A and quenched by red light, and has been ascribed to the reduction of the PQ pool via cyclic ferredoxin:plastoquinone reductase activity. It is often used as an assay for investigating processes postulated to involve cyclic electron flow around photosystem I (CEF1).

We have investigated the basis of the $F_{\text{rise}}$ in spinach chloroplast preparations, observing a similar antimycin A-sensitive phenomenology under conditions where Q$_{A}$-associated PSI1 variable fluorescence has been eliminated due to the presence of hydroxylamine and DCMU. Therefore the signal does not reflect PQ reduction associated with CEF as previously supposed, but another process.

The $F_{\text{rise}}$ is abolished in the presence of diphenyleneiodonium, an FNR inhibitor. Examination of the fluorescence induction kinetics indicates reduction of the bulk PQ pool by NADPH, although the $F_{\text{rise}}$ is unaffected by saturating concentrations of the b6f Q$_{b}$ inhibitor tridecystigmatellin and the kinetics of intersystem chain oxidation by P700$^{+}$ in the presence of NADPH and Fd are slower than those observed in the $F_{\text{rise}}$ assay. We conclude that the fluorescence quenching species participating in the $F_{\text{rise}}$ is not PQ or a component linked to the PQ pool. Redox titration of the rise suggests the presence of a component with a midpoint potential of approximately $-340$ mV.

We suggest that the quencher removed during the $F_{\text{rise}}$ assay is a photosystem II (or antenna)-associated, and may represent a low potential variant of QA within a subpopulation of this enzyme, or a conformational change associated with a PSII or antenna redox carrier. As such, the $F_{\text{rise}}$ may not be directly associated with CEF1, but may represent the activation of a redox switch for regulation of CEF1 or other processes.

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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 (0–60 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