ALTERED LIPID MATRIX CHANGES ENERGY DISTRIBUTION IN THYLAKOIDS.

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INTRODUCTION

Photosynthetic membranes differ from other cellular membranes in that they have a thylakoid specific lipid composition and a high degree of polyunsaturated fatty acids. The typical acyl lipid complement of thylakoids seems to have two functions: (i) organization of the membrane matrix in which the pigment protein complexes are embedded; (ii) indirect structure-function relationships between lipid matrix and pigment protein complexes which influence energy distribution behaviour. The role of high amounts of polyunsaturated fatty acids and indirect structure-function relationships can be studied by inhibiting fatty acid desaturation sequence with pyridazinone herbicides. Depending on the pyridazinone-type used, electron transport, carotenoid synthesis or fatty acid desaturation may be influenced. The pyridazinone 4-chloro-5-(dimethylamino)-2-phenyl-3(2H)pyridazinone (BASF 13-338=SAN 9785), a weak PS II electron transport (1) and potent state change inhibitor (2) has little effect on the organization of pigment forms. However, it inhibits different desaturation steps in the synthesis of polyunsaturated fatty acids leading to an increase of appressed/nonappressed regions of thylakoids (3).

MATERIALS AND METHODS

Petunia hybrida plants were treated with a solution of 160 μ M pyridazinone for 7d. Low temperature fluorescence kinetic measurements of leaf discs were carried out using excitation light of 633 nm (HeNe laser, 20 W/m²). Fluorescence signals measured at 695 nm (PS II) and 735 nm (PS I) were digitized on line (IMSAI 8080 microcomputer) and stored in core memory for data handling. The characteristics of energy distribution, i.e. trapping T, grouping G, spillover S and dissipation D were analysed according to the Energy Flux Theory (4). Thylakoid lipids were isolated by TLC and fatty acids were analysed by GC.

RESULTS

Fatty acid changes during pyridazinone incubation period. Whereas the qualitative and quantitative lipid complement as well as the chlorophyll content of thylakoids remain constant (data not shown), changes in the fatty acid composition during the pyridazinone treatment have been observed. The pyridazinone alters

II.13.714

the unsaturated character of thylakoid membranes by decreasing mainly linolenic acid and increasing the content of saturated, mono- and di-unsaturated C₁₆- and C₁₈-fatty acids. This leads to a drastic decrease of double bond indices (Fig.1). These effects are most pronounced in glycolipids (MGDG, DGDG, SQDG) and less in the phospholipid fractions (PG, PL).

Fig.1: Fatty acid composition of monogalactosyl-diglyceride (MGDG) after 7 d treatment with the pyridazinone herbicide. o Control; ● 160 μ M BASF 13-338 (SAN 9785)

Low temperature fluorescence kinetics. 77 K fluorescence kinetics indicate a loss in the variable fluorescence $F_2(v)$ and $F_1(v)$, a decrease in $F_1(M)$ and an increase in the initial fluorescence $F_2(0)$ (Fig.2). The ratio $F_2(v)/F_2(M)$, which has been shown to be proportional to the trapping probability p_{2b} (5), decreases from 0.75 to 0.47, whereas αN , which implies how much energy in PS I originates from its own light absorption remains nearly constant. According to (4) the F_1 vs F_2 -plots yield straight lines with the same slopes indicating that spillover is not affected by an altered lipid matrix. At any time when grouping is zero ($k=0$, $m_s=K/(1-K)$, K , the temperature constant for interactions of RCs with internal acceptors (pheophytin, Q_A) can be calculated from the V vs V/s -plots and has the value.145. V means the normalized variable fluorescence; s means the area growth curve i.e. the non-fluorescence.

Fig.2: Simultaneous fluorescence kinetics of PS I (F_1) and PS II (F_2) at 77 K. Determination of K . A or o = Control; B or ● = 160 μ M BASF 13-338 (SAN 9785)

Fig.3: Calculation of grouping probabilities p_{22} from V_i of EADIE-HOFFSTEE-plots (V vs V/s); $k=(F_2(v)/F_2(0))p_{22}$; $K=0.145$

The curvature of the experimental signals contains the information for grouping of neighbouring PS II-LHC-units. Therefore, the grouping probabilities p_{22} can be calculated from the V_i of the EADIE-HOFFSTEE-plots (V vs V/s) (Fig.3).

The effect of an altered lipid matrix on energy distribution can be shown in efficiency terms p_{ij} , and in R_{ij} relative distances between the pigment protein complexes depending on the time of treatment with the pyridazinone. Fig.4 shows that the quantum yields p_{ij} for all energy fluxes as well as the relative distances R_{ij} are affected. There is a good statistical correlation between alterations in quantum yields p_{ij} and alterations in the fatty acid composition with significancies of 0.1 to 5 %.

Fig.4: Energy distribution terms p_{ij} and relative distances R_{ij} , where p_{ij} gives the quantum yield of an energy flux E_{ij} ($E_{ij}/\text{absorption of PS I or PS II}$). R_{ij} represents the relative distances between the pigment protein complexes i and j calculated from the rate constants using the FÖRSTER-resonance-relationship: $R_{ij}=k^{-1/6}$.

DISCUSSION

It has been proposed that the shape of a given lipid molecule is determined by the ratio of polar headgroup and the type of acyl moieties (6). Therefore, lipid packing properties are expected to bring about asymmetrical distribution of lipid classes and lipid species in membranes. The pyridazinone induces an altered ultrastructure of thylakoid membranes by increasing the ratio of appressed/nonappressed regions in grana. This effect may be due to changes in fatty acid composition, from a more unsaturated (high linolenic acid content) to a more saturated (lower linolenic acid content) state of the thylakoid lipid matrix. The frequency of linolenic acid/linolenic acid-species in glycolipids is lowered by nearly 50 %.

Energy transfer between different pigment protein complexes depends on close distances and could be influenced by the lipid

II.13.716

matrix. Calculations in (7) suggest, that in appressed regions there is a minimum set-up of lipids which favours energy transfer between pigment protein complexes. Our results indicate a strong correlation between grouping of PS II-LHC-units and the content of linolenic acid in glycolipids.

Considering the Lateral Segregation Theory (7), we assume, that due to alterations in shape, glycolipids, especially MGDG, are inserted into appressed rather than nonappressed regions of thylakoids. Therefore, we propose the following model for pyridazinone effect on thylakoids:

The Model. Data calculated from low temperature fluorescence kinetics and compiled in a synergetic way demonstrate energy distribution constellation in thylakoids after 7 d treatment with the pyridazinone. The models show a thylakoid sac originating from different grana stacks, respectively. The structure of the lipid matrix is related to energy distribution in a grouped bipartite model (pipeline model, 8).

Fig.5: Grana-, thylakoid- and energy distribution models.

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