Temperature — Jump Chlorophyll Fluorescence Induction in Plants

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In contrast to slower heating rates, a temperature jump reveals complex rise phases in the heat induced chlorophyll fluorescence emission increase in intact plants. Three rise phases have been detected which indicate the stepwise loss of different quenching mechanism of system II fluorescence. Two of the phases appear to reflect heat deactivation of the system II reaction centers, while the other may be associated with the induction of hydrogenase activity. Variations in T_{max} of the jump, for the increase in different plant varieties, suggest a correlation with membrane lipid phase transitions affecting thylakoid membrane structure and the fluorescence increase.

Chlorophyll fluorescence has been a valuable indicator for the state and functioning of the photosynthetic apparatus (for a review, *cf.* ref. 1). Changes in chlorophyll fluorescence yield, which reflect changes in energy conversion efficiency at System II centres, can be induced in three ways; a. by light, resulting in a transient exhaustion of electron acceptors (Kautsky effect [2]), b. by anaerobic conditions in darkness [3–5], due to the reduction of System II acceptor Q [6] by an endogenous electron donor, and c. by heating to 45 – 55 °C in the dark [4, 7, 8], in which case nothing is known about the mechanism inducing an observed fluorescence increase.

We have studied the heat induced fluorescence increase by means of a temperature jump (*T*-jump: 95% of a 30 °C rise in 25 s), rather than with the slow heating rates (approx. 1 °C/min) used in previous studies. Details concerning the measuring apparatus will be communicated in a following publication. Fig. 1 a shows a typical *T*-jump (20° to 49 °C) fluorescence induction curve in the green unicellular alga *Scenedesmus obliquus*. There is a surprising complexity of at least three rise phases, designated here as α, β, and γ, reflecting different heat induced processes, which result in loss of fluorescence quenching. These processes obviously

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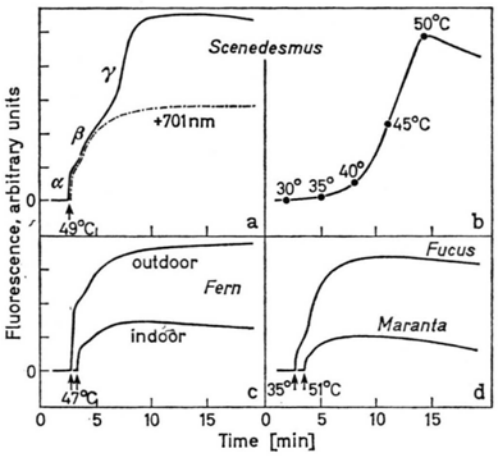

Fig. 1. Heat induced changes of chlorophyll fluorescence yield in plants. a. *T*-jump curves (20 to 49 °C) in *Scenedesmus obliquus*; the greek letters designate different rise phases; dotted curve in 701 nm light (30 μW/cm²) interrupted for 10 ms every second to measure fluorescence during a blue measuring flash (5 μW/cm²). b. Conventional heating curve in *Scenedesmus* at approx. 1.6 °C/min. c. Comparison of *T*-jump curves (20 to 47 °C) of two ferns, *Polistichum munitum* collected outdoors, and *Nephrolepis sp.* grown indoors. d. *T*-jump curves in the marine phaeophyte *Fucus* (for 15 to 35 °C) and in a potted plant of tropical origin, *Maranta* (for 15 to 51 °C). All curves, except for the dotted line in a., were recorded in a weak broad band blue beam (0.2 μW/cm²), which by itself did not affect fluorescence yield. Fluorescence was measured at wavelengths >675 nm.

are not instantaneous but have time constants of from fractions of a second to several minutes. Comparison with a "conventional" heating curve (see Fig. 1 b) reveals that with slow heating most of the information relating to the stepwise deactivation of different quenching mechanisms is lost. The dotted line in Fig. 1 a shows the curve recorded in far-red light (interrupted only during short measuring flashes). Far-red light preferentially excites Photosystem I and keeps quencher Q in the oxidized state. There is a complete suppression of the γ-phase, whereas α and β are practically unaffected.

Quenching of System II chlorophyll fluorescence depends on the integrity of the reaction center complex ZPQ; Z must be in a state to donate an electron, Q in a state to accept this electron, and P in a state to sensitize the transfer. As can be concluded from Fig. 1 a, quenching is removed during γ due to reduction of Q by some endogenous donor. The intensity of the measuring beam in this experiment is too low to cause appreciable reduction by Z. From the work of Döring *et al.* [9] it can be assumed that P is not deactivated at 48 °C. On the other hand Z, a component of the water splitting enzyme system, is deactivated in the region of 45 – 50 °C [9, 10]. Pos-

sibly α, β are expressions of the deactivation of the water-splitting enzyme system.

We have obtained T-jump fluorescence induction curves for a variety of plants, all of which show α and β phases, while only a few have a pronounced γ phase as in *Scenedesmus*. A preliminary survey suggest that the γ phase is correlated with hydrogenase activity [11, 12], present only in some plant species.

The T_{max} required for appreciable fluorescence increases is dependent on the previous thermal environment of the plant as well as its genetic history. Fig. 1 c compares T-jump curves (20 to 47 °C) in a wild fern, *Polystichum munitum* collected outdoors, having survived winter temperatures around 0 °C, and a potted indoor fern, *Nephrolepis sp.* growing at approx. 21 °C. Both ferns showed typical Kautsky effects indicating normal photosynthetic activity. Their responses on heating differ markedly; the rise in the outdoor plant is far more rapid and reaches a much higher level. In Fig. 1 d T-jump curves of the marine phaeophyte, *Fucus* (maintained at approx. 7 °C) and of a potted plant of tropical origin, *Maranta leuconeura*, are depicted. Whereas *Fucus* shows a remarkable fluorescence increase at 35 °C, even at 51 °C the rise in the tropical plant is small.

If there is a correlation between α, β phases of the T-jump curves and deactivation of the water-splitting enzyme system, as suggested above, it is difficult to explain the wide range of deactivation temperatures by differences in the enzyme system itself. We propose that the variability is due to the particular composition of the thylakoid membrane, on which the enzyme system is located and dependent upon for its integrity in each particular plant. It is known that lipid composition, especially degree of unsaturation and fatty acid chain lengths, is highly correlated to the thermal growth environment [13, 14]. Lipids undergo phase transitions from a fluid-crystalline to a fluid state, the importance of which for some biological membranes has been demonstrated [15, 16]. Lipid phase transition temperatures are in the range where T-jump fluorescence induction occurs in plants. A reasonable working hypothesis then is that the α, β phases of the T-jump curves reflect a phase transition of the lipid component in the thylakoid membrane, which leads to inactivation of the water splitting enzyme system. The γ-phase may also reflect a phase transition assuming this leads to decreased O_2 solubility in the membrane lipids, including Q and plastoquinone, to such an extent that the endogenous hydrogenase is activated.

T-jump fluorescence induction curves obviously contain valuable information concerning the state of the photosynthetic apparatus and presumably the watersplitting system along with its membraneous environment. Further studies, now underway, of this phenomenon may substantiate the working hypothesis stated above. It is foreseen that T-jump fluorescence curves can be useful as a rapid and relatively easy test for temperature sensitivity of any plant variety.

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