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THE INFLUENCE OF THE ELECTRIC DIFFUSION POTENTIAL ON DELAYED FLUORESCENCE LIGHT CURVES OF CHLOROPLASTS TREATED WITH 3-(3,4-DICHLOROPHENYL)-1,1-DIMETHYLUREA

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Summary

The potassium salt-induced transient increase of delayed fluorescence yield was studied in pea chloroplasts treated with 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

A simple kinetic model is proposed to account for the actinic light intensity dependence of the delayed fluorescence enhancement by the transmembrane diffusion potential induced by sudden salt addition. The electric field dependence of the rate constants for the recombination of primary separated charges with and without subsequent electronic excitation of reaction center chlorophyll was obtained.

From the value of enhancement of delayed fluorescence by salt concentration gradients at saturating actinic light intensity, it is concluded that the distance, normal to thylakoid membrane surface, between the primary acceptor and the donor of Photosystem II is smaller than the membrane thickness.

Introduction

The delayed fluorescence of chlorophyll *a* in green plants originates from the recombination of primary separated charges in the reaction center of Photosystem II [1]. The intensity of the delayed emission depends on electric potential [2] and proton concentration gradient [3] across the thylakoid membrane.

It has been postulated that the development of the proton electrochemical

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Chl, chlorophyll; PS, Photosystem.

gradient across the membrane may lead to the reduction of the activation energy of the recombination.

Barber [2] has introduced a convenient method for studying the effect of the transmembrane electric potential on delayed fluorescence by a sudden addition of salts to chloroplast suspension.

The elucidation of the mechanism of the electric field influence on delayed fluorescence is, however, complicated because of the multiphase kinetics of the delayed light in active chloroplasts and because of possible salt effects on electron transport, electrochemical proton gradient and, consequently, on parameters of delayed fluorescence. In the present work, the effect of the diffusion potential on delayed fluorescence was studied in chloroplasts with DCMU-inhibited electron transport. In such chloroplasts, the kinetics of the delayed fluorescence decay on the millisecond time scale exhibit a single component with $t_{1/2} \approx 500$ ms.

With the help of a simple kinetic model for delayed fluorescence in DCMU-inhibited chloroplasts it appeared to be possible to obtain the electric field dependence of the rate constants of the recombination reactions with and without electronic excitation of reaction-center chlorophyll. From the extent of the enhancement of the delayed light emission in the presence of the diffusion electric potential, the distance between the primary acceptor and the donor of Photosystem II has been estimated. Part of this work was recently presented in Ref. 4.

Materials and Methods

Chloroplasts were prepared by the method of Arnon [5] from leaves of pea plants grown in a growth chamber. The chloroplasts were washed once in a medium containing 10 mM sodium phosphate/potassium phosphate buffer (pH 7.8) and 0.4 M sucrose. The concentration of K^+ was 1 mM. Chloroplasts were stored in a small volume of the washing medium at 0°C and before measurement were diluted with the same medium to give a concentration of 20 μg Chl/ml. Delayed fluorescence was measured with a conventional rotating cylinder phosphoroscope [6] 1.25 ms after actinic light illumination. Light was provided from a 300 W incandescent lamp with a red cut-off filter (λ>620 nm). $5 \cdot 10^{-5}$ M DCMU were added to 0.5 ml of the chloroplast suspension and the delayed fluorescence was measured under illumination with the actinic light attenuated to a desired intensity by calibrated neutral filters.

0.1 ml of a salt solution was rapidly injected into the measuring cuvette after the steady-state level of delayed light intensity had been attained.

Results

Fig. 1 shows typical delayed fluorescence bursts induced by addition of K^+ salts into a chloroplast suspension in the presence of DCMU. Because of the high cationic permeability of the thylakoid, its interior becomes electrically more positive with respect to the ambient solution. This leads to an enhancement of the delayed light emission immediately after salt injection $-L(\psi_m)$. A high salt concentration in the reaction mixture does not change the charac-

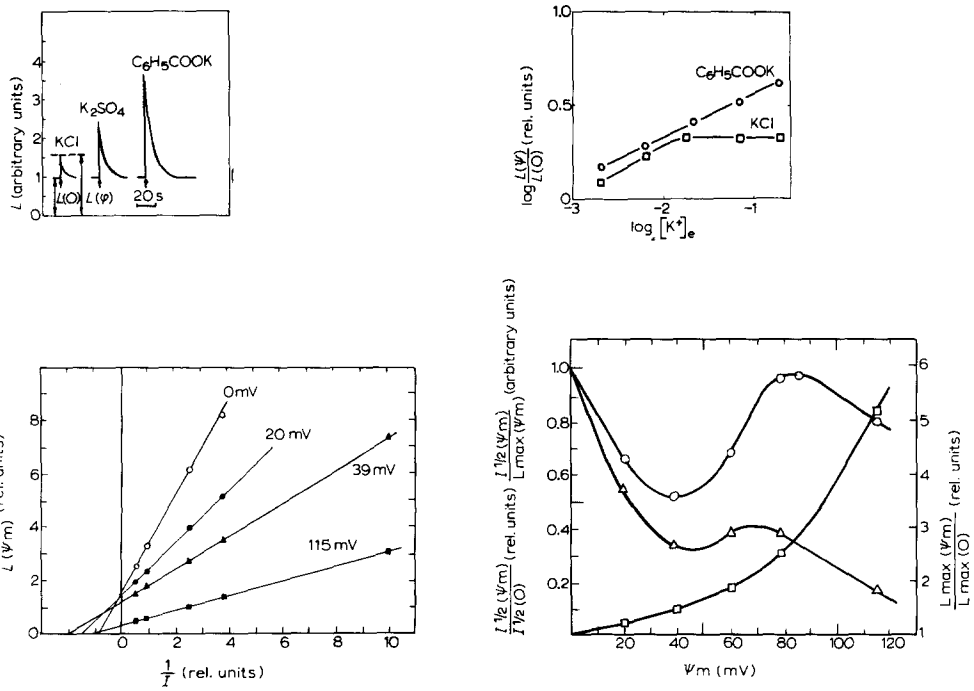

Fig. 1. Bursts of delayed fluorescence after addition of various potassium salts. 0.1 ml of 0.5 M salt solution was added at the time indicated by arrows to 0.5 ml chloroplast suspension containing 10 mM phosphate buffer, 400 mM sucrose, 10^{-5} M DCMU and chlorophyll at 20 μg/ml. Initial concentration of K^+ was 1 mM; actinic light intensity was 10 000 erg/cm^2 per s.

Fig. 2. The extent of delayed fluorescence stimulation by salt addition. $L(0)$ is delayed fluorescence before and $L(\psi)$, after salt addition (see Fig. 1). Except for added salt concentration, experimental conditions were as in Fig. 1.

Fig. 3. Double reciprocal plots of C_6H_5COOK-stimulated delayed fluorescence intensity, $L(\psi_m)$, as function of actinic light intensity. Transmembrane electric diffusion potentials calculated from Eqn. 9 are indicated at curves. Experimental conditions were as in Fig. 1.

Fig. 4. Relative values of stimulated emission of DCMU-treated chloroplasts, $L(\psi_m)/L(0)$ (squares), half saturating actinic light intensity $[I_{1/2}(\psi_m)]/[I_{1/2}(0)]$ (circles), and ratio $[I_{1/2}(\psi_m)]/[L_{max}(\psi_m)]$ (triangles), as function of transmembrane electric diffusion potential.

teristics of the emission, since its intensity always drops to the initial level — $L(0)$, 10—15 s after salt addition.

The extent of the delayed fluorescence stimulation, $L(\psi)/L(0)$, at the maximal actinic light intensity is shown in Fig. 2 as a function of a salt concentration. The amplitude of bursts is saturated at $5 \cdot 10^{-2}$ M KCl. In the presence of potassium benzoate the saturation is not attained up to $1 \cdot 10^{-1}$ M because of a low permeability of thylakoids for benzoate anion.

Plots of $L(0)$ and $L(\psi_m)$ versus actinic light intensity in reciprocal coordinates are stright lines (Fig. 3). Cross points of the plots with the axes yield the values of L_{max} (the delayed fluorescence at saturating actinic light) and $I_{1/2}$ (half-saturating intensity of actinic light).

Relative parameters of light curves of the delayed fluorescence $L_{max}(\psi_m)/L_{max}(0)$ and $I_{1/2}(\psi_m)/I_{1/2}(0)$ as the functions of electric transmembrane potential — ψ_m are shown in Fig. 4. One can see that L_{max} increases monotonously with electric potential, and $I_{1/2}$ has a minimum at ψ_m = 40—50 mV.

Discussion

Denoting the reaction center chlorophyll of Photosystem II, primary acceptor and water-splitting system as P, Q and Z, respectively, PS II photoreactions in DCMU-treated chloroplasts can be written as:

$$Z \rightleftharpoons P \rightleftharpoons Q$$

Transitions between different redox states of this complex of electron carriers can be presented as follows:

$$Z^1P^1Q^O \underset{k_{f1}+k_d}{\overset{k_2}{\rightleftharpoons}} Z^1P^*Q^O \underset{k_r^*}{\overset{k_{ph}}{\rightleftharpoons}} Z^1P^OQ^1 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Z^OP^1Q^1 \qquad (1)$$

$$\uparrow \quad \_\_\_\_\_ \quad k_r \quad \_\_\_\_\_ \quad \uparrow$$

where Z^1, P^1 and Q^1 are carriers in the reduced state and Z^O, P^O and Q^O in the oxidised state; P^* is the reaction center chlorophyll in the excited state; k_{-1}/k_1 is the equilibrium constant of the electron transport between Z and P; k_2 is the rate constant which changes linearly with actinic light intensity; k_r^* and k_r are the rate constants for recombination reactions with and without electronic excitation of P; k_{ph}, k_{f1} and k_d are the rate constants for photochemical, radiation and radiationless deactivation of the excited P, respectively.

The steady-state intensity of the delayed fluorescence from a unit volume, (L) is:

$$L = N \cdot \varphi_{f1} \cdot k_r^* \cdot \frac{k_{-1}}{k_1} (Z^OP^1Q^1) \qquad (2)$$

where N is the concentration of reaction centers, φ_{f1} is the quantum yield of chlorophyll fluorescence and $(Z^OP^1Q^1)$ is the steady-state probability of $Z^OP^1Q^1$. It can be solved from Eqn. 1. Assuming k_r^*, k_r, $k_{f1} + k_d << k_{ph}$ and $k_{-1}/k_1 << 1$, $(Z^OP^1Q^1)$ can be written in the form:

$$(Z^OP^1Q^1) = \frac{k_2}{k_2 + \left(k_r + \frac{k_{f1} + k_d}{k_{ph}} k_r^*\right) \frac{k_{-1}}{k_1}} \qquad (3)$$

Hence, the delayed fluorescence is a hyperbolic function of actinic light intensity

$$L = L_{max} \cdot \frac{k_2}{k_2 + I_{1/2}} \qquad (4)$$

where the delayed fluorescence at saturating actinic light intensity is:

$$L_{max} = N \cdot \varphi_{f1} \cdot k_r^* \frac{k_{-1}}{k_1}, \qquad (5)$$

and the half-saturating actinic light intensity is

$$I_{1/2} = \frac{k_{-1}}{k_1}\left(k_r + \frac{k_{f1} + k_d}{k_{ph}} k_r^*\right) \tag{6}$$

By changing energy levels of reacting species, the diffusion potential across the thylakoid membrane can influence the delayed fluorescence intensity, if the reacting species are charged and properly oriented in the membrane. The effect of the electric potential (ψ) on L_{max} may be due to changes in activation energy of the process labelled k_r^* [3] and/or to changes in the equilibrium constant k_{-1}/k_1. Therefore, L_{max} can be written as an exponential function of ψ [3]:

$$L_{max}(\psi) = N \cdot \varphi_{f1} \cdot k_r^*(0) \cdot \frac{k_{-1}(0)}{k_1(0)} \cdot \exp \frac{\psi F}{RT} \tag{7}$$

where $k_r(0)$, $k_1(0)$ and $k_{-1}(0)$ are the rate constants in the absence of electric field. The exponential dependence of the delayed fluorescence on ψ was experimentally proved by Barber [7].

Keeping in mind that only that fraction $\alpha \leqslant 1$ of the membrane potential ψ_m which is applied to separated charges enhances L_{max}, an experimental transmembrane potential dependency of L_{max} can be written as:

$$\frac{L_{max}(\psi_m)}{L_{max}(0)} = \exp\left(\frac{\alpha \psi_m F}{RT}\right) \tag{8}$$

where $L_{max}(\psi_m)$ and $L_{max}(0)$ are L_{max} in the presence and absence of electric field, respectively; R, T and F are conventionally assigned.

Combining Eqn. 8 with Goldman's equation

$$\psi_m = \frac{RT}{F} \cdot \ln \frac{K_e + \beta A_i}{K_i + \beta A_e} \tag{9}$$

where $\beta = P_a/P_k$ is the ratio of the anion and cation permeabilities of the membrane; K and A are cation and anion concentrations, respectively; subscripts 'i' and 'e' refer to the internal and external solutions, respectively, one can obtain:

$$\frac{L(\psi_m)}{L(0)} = \exp\left(\alpha \cdot \ln \frac{K_e + \beta A_i}{K_i + \beta A_e}\right) = \left(\frac{K_e + \beta A_i}{K_i + \beta A_e}\right)^{\alpha} \tag{10}$$

At low salt concentration, the stimulation of the delayed fluorescence is determined mainly by the K_e/K_i ratio:

$$\log \frac{L(\psi_m)}{L(0)} = \alpha \log \frac{K_e}{K_i} \tag{11}$$

and α is the slope of the double logarithmic plot of the delayed fluorescence enhancement as function of salt concentration (Fig. 2).

For chloroplasts with DCMU, Eqn. 11 has been previously deduced by Mar and Roy [8] who obtained $\alpha = 1.36$ from data of Barber and Kraan [2] on salt stimulation of the delayed fluorescence of the uninhibited chloroplasts. To apply this relation to chloroplasts without DCMU, the arbitrary assumption

was made that the rate constant for reaction of Q with the next intermediate in the electron-transport chain varies with membrane potential in the same way as the recombination rate. The unrealistic value obtained may be also due to salt concentration effects aforementioned in the Introduction.

We obtain $\alpha = 0.3$ with KCl and $\alpha = 0.24$ with benzoate.

In DCMU-inhibited chloroplasts, the 0.5 s delayed fluorescence is generated by the electron transfer from the primary acceptor to the donor side of Photosystem II. Therefore, the fraction α of the membrane potential effective in stimulation of the delayed light is applied across this distance — the separation between Z and Q.

Assuming the hydrophobic layer of the thylakoid membrane to be a homogenous dielectric of 40 Å thickness [9], this distance is estimated as being 10—12 Å. This value is smaller than an estimate of distance between recombining charges (20—25 Å) made by Ortoidze and colaborators [10] from an external electric field stimulation of delayed fluorescence in dried chloroplasts films, but coincides with the value obtained by Jursinic and co-workers [11] from a salt stimulation of millisecond-delayed fluorescence. In the latter work, the stimulation of the delayed light by electric field was observed after the development of the transmembrane proton concentration gradient only. In our experiments with DCMU-inhibited chloroplasts and dried films of chloroplasts the stimulation occurred even without transmembrane proton gradient.

Values obtained from the delayed fluorescence stimulation experiments are inconsistent with a simple picture in which electron carriers have fixed positions at opposite sides of the thylakoid membrane. Because of a possible inhomogeneity of dielectric properties of the membrane the values obtained are only a rough estimation. Nevertheless, they clearly show that the distance between recombining charges does not bridge the entire membrane thickness.

The location of Z and P on its inside and Q on its outside is evidenced by simple and fast kinetics of 515 nm absorbance changes [12] and by the fact that proton release coupled to the production of oxygen occurs toward the inside of the membrane [13]. There are, however, results indicating that P [14], or Z [15] may be located near the outer surface of the membrane. Of particular relevance are the results of Giaquinta and Dilley [16], that the oxygen-evolving site releases its protons toward the outside of the thylakoid membrane in the presence of DCMU, with silicomolibdate as electron acceptor.

Another electric field effect is on k_r. In fact, the ratio:

$$\frac{I_{1/2}}{L_{max}} = \frac{1}{\varphi_{f1} N} \left(\frac{k_r}{k_r^*} + \frac{k_{f1} + k_d}{k_{ph}} \right) \tag{12}$$

changes with ψ_m (Fig. 4). The recombination reaction which produces delayed fluorescence apparently proceeds at a small rate compared to that of the recombination without exciton production [17], i.e.,

$$k_r > k_r^* \frac{k_{fl} + k_d}{k_{ph}}$$

This means that the electric field effect on the ratio $I_{1/2}/L_{max}$ is due to changes in k_r/k_r^* and that k_r and k_r^* are different in electric field dependence, with the

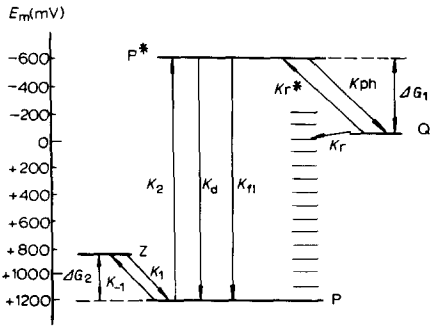

Fig. 5. Energy level scheme for delayed fluorescence of DCMU-treated chloroplasts (see text).

minimal value of k_r at ψ_m being 40 mV. The difference in electric field effects on k_r^* and k_r, apparently reflect different mechanisms of radiation and radiationless recombination of primary separated charges. A hypothetical scheme of electron transitions in the primary redox couple of the reaction center of Photosystem II is shown in Fig. 5.

In terms of this scheme, a quantum of the delayed fluorescence is emitted with the transition of an electron from the primary acceptor to the singlet excited level of chlorophyll P*. This requires an activation energy ΔG_1. Whenever there exists a transmembrane potential and it is positive inside, the transition occurs with a lower activation energy.

Radiationless recombination is a slow electron tunnelling from the primary acceptor to the ground electron level of chlorophyll, which is accompanied by a vibrational excitation of a chlorophyll molecule followed by a fast vibrational relaxation. The rate of tunnelling depends on the position of the electron level of the acceptor with respect to vibrational levels of ground electronic state of the chlorophyll. This position is changed by the electric field and minimal k_r (see Fig. 4) value corresponds to the acceptor level situated between neighbouring vibronic levels of the chlorophyll.

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