ANTAGONISTIC EFFECT OF MONO- AND DIVALENT-CATIONS ON LIFETIME (τ) AND QUANTUM YIELD OF FLUORESCENCE (Φ) IN ISOLATED CHLOROPLASTS

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1. Introduction

Several recent publications [1–3] describe the effects of cations on chlorophyll *a* fluorescence in sucrose-washed chloroplasts. In these cation-free chloroplasts, NaCl (10 mM) decreases the fluorescence of the *'O'* (initial) and *'P'* (maximum) levels [2]. The addition of $MgCl_2$ (10 mM) at 298°K re-establishes the fluorescence intensity of 685 nm and 695 nm bands (F685 and F695, respectively) compared to the 735 nm band (F735) at 77°K. Attributing F685 and F695 to system II and F735 to System I (see ref. [4]) it was concluded [1] that a modification in the distribution of the excitation energy occurs in favor of System I, when NaCl is added. On the other hand, the addition of $MgCl_2$ leads to a decrease of energy received by System I and an increase in energy received by System II [5]. The cations are believed to modify the distribution of excitation energy between the two systems by modifying the 'spill-over' of this energy from System II to System I.

The object of the experiments described here was to verify whether the variations in fluorescence intensity (F) induced by both the mono- and divalent-cations correspond with the modifications of the quantum yield of fluorescence as calculated from the lifetime measurements

$$\tau = \Phi\tau O$$

where τ = lifetime of fluorescence, Φ = quantum yield of fluorescence and τO = intrinsic lifetime of fluorescence depending upon the characteristics of the absorption band. This information is essential to the understanding of the salt-effects in terms of the changes in the absorption cross-section or in the energy-transfer (see ref. [6]). A correspondence between the changes in F and τ had been shown only for the effect of Mg^{2+}-cations on isolated chloroplasts [6,7]. The concept of changes in energy-transfer was confirmed, but beside this change of Photosystem II deactivation, Butler and Kitajima [8] and Loos [9] showed that slight variations of direct sensitization of each photosystem are induced by cation concentration changes. We present here data on the relation between τ and F during the fluorescence induction in three different cases: cation-free sucrose-washed chloroplasts, sucrose-washed chloroplasts plus NaCl and, the same plus further addition of $MgCl_2$. Antagonistic effect of mono- and divalent-cations on τ (and, therefore on true, Φ) of chlorophyll *a* fluorescence in chloroplasts are reported here. These data have confirmed the correspondence between fluorescence intensity and fluorescence yield in all these cases and have provided further indications of the possible modifications of excitation energy transfer between photosynthetic units of System II.

2. Materials and methods

Lifetime measurements were made with a phase fluorimeter, a complete description of which will soon be published (I. Moya, in preparation). The light source was a He–Ne laser (λ = 6328 Å) giving a light intensity of 4×10^4 ergs cm^{-2} s^{-1}; the frequency of

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modulation was 14.5 MHz. A stop-flow system was used to allow variation of fluorescence intensity from the *O*- to the *P*-level. Simultaneous measurements on τ and F were made. Each set of recorded point was the result of 32 consecutive kinetics averaged in a multichannel analyser. The sample was placed in a 2 × 2 mm optical-path cell and fluorescence was observed at 90° through a Schott RG5 filter transmitting the entire emission band of chlorophyll *a*.

The chloroplasts of lettuce or peas were prepared by the method described by Gross [10], but were washed three times in cation-free medium before use. Such chloroplasts have extremely low concentrations of cations [3]. The chlorophyll concentration of our samples was approximately 25 μg/ml suspension.

3. Results

First, $\tau = f(F)$ measurements were made with chloroplasts from lettuce, purchased from the market, but these were shown to be very little sensitive to the addition of salts. Figure 1 shows the results obtained with such chloroplasts (without salt-addition). The marked curvature (towards the positive τ, concave-curve) observed here can be easily interpreted if we suppose that a constant (to be referred as 'dead' emission) fluorescence is superimposed on the System II emission which follows the $\tau_{II} = \tau o \cdot \Phi_{II}$ law during fluorescence induction (τ_{II} and Φ_{II} are lifetime and quantum yield of fluorescence of System II) (see ref. [11]). With this hypothesis, it is possible to compute from fig.1, the intensity and lifetime of this dead fluorescence. Analysis of the original values into the values for the above mentioned components is very sensitive to errors. However, a dead fluorescence with a lifetime close to 4.5 ns and an intensity of 10–15% of the *O*-level qualitatively explains our results. This dead fluorescence can arise from chlorophyll molecules disconnected from their in situ state in the membrane during extraction of chloroplasts or may have existed as such in the native state. Paschenko et al. [12], in their experiments with pea chloroplasts, have also observed a component with a lifetime of 4.5 ns, while following the decay of fluorescence after excitation with a 10 ps laser pulse. Our result agrees with theirs.

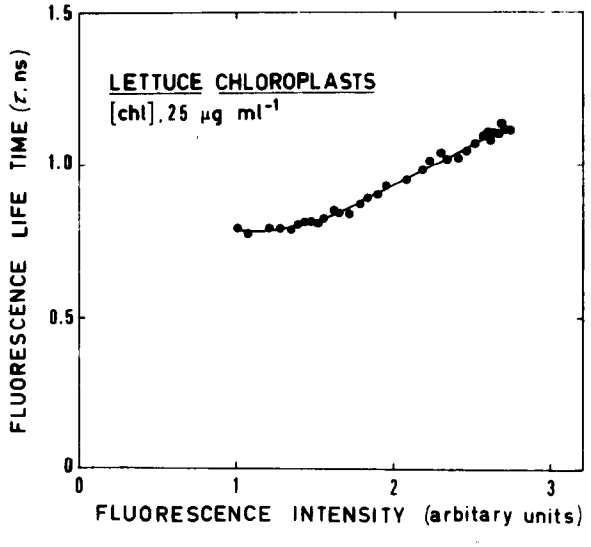

Fig.1. Lifetime of chlorophyll *a* fluorescence (τ in nanoseconds) as a function of fluorescence intensity (F, in arbitrary units) in lettuce chloroplasts. Fluorescence intensity was varied by the stop-flow technique of Lavorel. See text for details.

It is highly unlikely that the above mentioned dead fluorescence arises from System I because the τo value showed in fig.1 (0.84 ns) is more than two times larger than the value obtained in algae [13] or in chloroplast preparations and we know that the quantum yield (and hence, τ) of System I is lower than that of System II [14]. We emphasize that in experiments on salt-effects, such chloroplasts, as described above, should be avoided because of the presence of 'disconnected' (or dead) chlorophyll *a* which mask the effects under study.

With chloroplasts from peas grown in our greenhouse this dead fluorescence did not appear and, therefore, these preparations were chosen for our salt-effect studies. (We note that the difference does not lie in the species of the plants used, but the precise physiological condition of the sample chosen.) Figure 2 shows a plot of fluorescence lifetime (τ in ns) as a function of fluorescence intensity (labelled fluorescence yield, Φ, because the intensity of excitation was constant, and, the 'dead' or 'disconnected' chlorophyll was absent). In cation-free chloroplasts, the τ-values ranged from 0.6–1.5 ns. An almost linear relationship was observed between τ and F.

The addition of 10 mM NaCl induced a strong decrease in τ at the *P*-levels and a smaller one at the

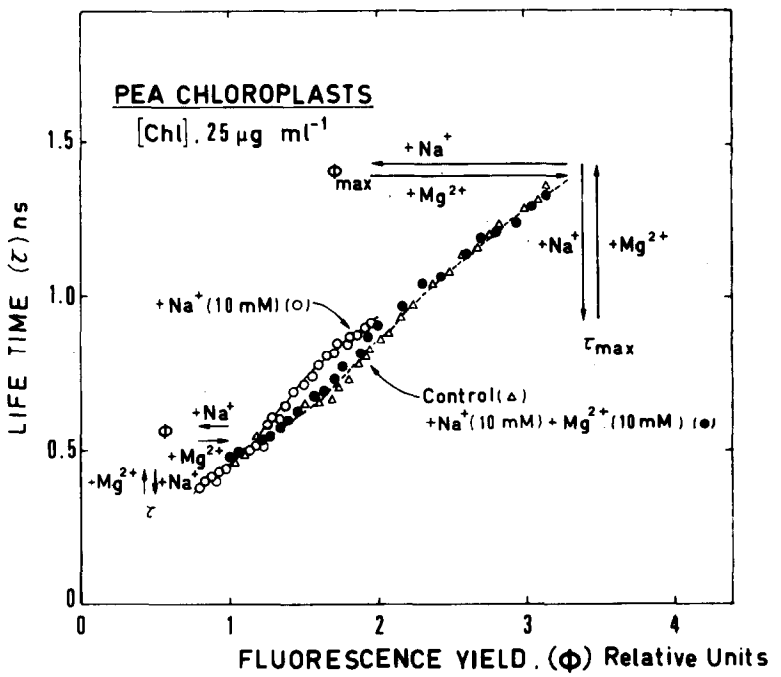

Fig. 2. Lifetime of chlorophyll *a* fluorescence (τ in nanoseconds) as a function of fluorescence intensity (labelled as fluorescence yield, Φ) in pea chloroplasts. Note antagonistic effect of Na^+ and Mg^{2+} on both τ and Φ at both *'O'* (labelled as τ and Φ) and *'P'* (labelled as τ_{max} and Φ_{max}) levels. Details as indicated on the figure and in the text.

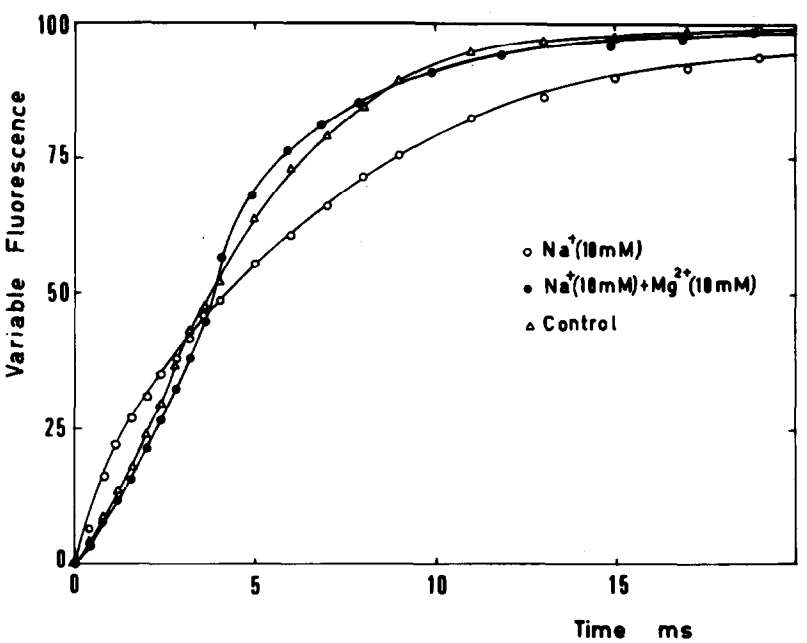

Fig. 3. Variable fluorescence in the presence of 10^{-5} M DCMU of 10 mM NaCl (○——○), 10 mM NaCl + 10 mM $MgCl_2$ (●——●) and sucrose washed (△——△) chloroplasts. The 3 induction-curves are normalized at the same maximum yield of fluorescence.

O-level. Parallel decreases in the fluorescence intensity at *O*- and *P*-levels were observed, and confirm the observations of Wydrzynski et al. [2] on spinach chloroplasts. The fact that τ and F are affected by the addition of NaCl proves that there are decreases in the quantum yield of fluorescence (both at *O*- and *P*-levels). The τ versus F curve shows a slight convex-curvature during the thermal-phase of fluorescence induction. An increase of the general slope is observed in the Na^+-chloroplasts compared to the control. Addition of 10 mM $MgCl_2$ to sucrose-washed chloroplast containing 10 mM NaCl restores strictly the original pattern of $\tau = f(F)$ relationship observed in control (sucrose-washed chloroplasts without any addition).

Figure 3 shows that in the presence of DCMU, the fluorescence induction rise is sigmoidal in sucrose-washed chloroplasts as in ($Na^+ + Mg^{2+}$) sample, whereas the induction is almost exponential in Na^+-plastids.

4. Discussion

The experimental conditions of the present work are different than those previously published [6] but, qualitatively, the effects of Mg^{2+} are the same. The new aspect of the present work lies in the investigation of the antagonistic effect of mono- and divalent-cations on τ and, therefore, on Φ of chlorophyll *a* fluorescence in chloroplasts. The basis of our interpretation is essentially that the constant (*O*-level) and the variable (*P*–*O*) fluorescence originate in the bulk (or antenna) chlorophylls of System II. This is consistent with the fact the τ/F ratios remain constant, in the first approximation, during the fluorescence induction in the case of algae and chloroplasts (in the absence of disconnected or 'dead' chlorophyll) (see refs [15,16]).

In sucrose-washed chloroplasts the spill-over rate constant is as low as in chloroplasts incubated in $Na^+ + Mg^{2+}$. The arguments for this statement emerge from the data presented here. The first one comes from the changes of the fluorescence yield of Photosystem II (τ–Φ). The fluorescence yield in the control, at the *O*-level ($\Phi^c(O)$) is expressed by:

$$[(\Phi^c(O)]^{-1} = (k_f + k_t + k_p) \cdot K_f^{-1} \quad (1)$$

where, k_f = rate constant of fluorescence deactivation = τo^{-1} (τo is natural lifetime), k_t = rate constant of deactivation by internal conversion and other processes and k_p = rate constant of photochemical deactivation.

On the other hand, fluorescence yield in the control at the *P*-level ($\Phi^c(P)$), where k_p is negligible, is:

$$[\Phi^c(P)]^{-1} = (k_f + k_t) \cdot k_f^{-1} \quad (2)$$

In the presence of Na^+, a further quenching appears in competition with the other deactivation pathways, and one must have:

$$[\Phi^{Na^+}(O)]^{-1} = (k_f + k'_t + k_p) \cdot k_f^{-1} \quad (3)$$

Where, k'_t is the changed k_t. Likewise:

$$[\Phi^{Na^+}(P)]^{-1} = (k_f + k'_t) \cdot k_f^{-1} \quad (4)$$

From the above equations, it follows that:

$$[\Phi^c(O)]^{-1} - [\Phi^c(P)]^{-1} = \frac{k_p}{k_f} = [\Phi^{Na^+}(O)]^{-1} = [\Phi^{Na^+}(P)]^{-1} \quad (5)$$

If $\Phi^c(O)$ is taken to be 1.0, one obtains, from data of fig.2:

$$[\Phi^c(O)]^{-1} - [\Phi^c(P)]^{-1} = 0.70 \pm 0.02 \text{ and}$$

$$[\Phi^{Na^+}(O)]^{-1} - [\Phi^{Na^+}(P)]^{-1} = 0.74 \pm 0.05.$$

The closeness of these values and the superimposition of the curves obtained in *O*-salt and ($Na^+ + Mg^{2+}$) samples suggests that, in the first approximation, the antagonistic action of Na^+ and Mg^{2+} on the fluorescence could be interpreted as a variation in the deactivation pathway(s) in competition with the fluorescence and photochemistry. One can explain the changes of this deactivation by a variation of spill-over rate constant. This conclusion is in agreement with the recent measurements of Govindjee and Wong [17] on changes in the degree of polarization of chlorophyll *a* fluorescence in sucrose-washed chloroplasts treated with NaCl and then $MgCl_2$.

The shape of the fluorescence inductions in the presence of DCMU are sigmoidal in *O*-salt and ($Na^+ + Mg^{2+}$) samples and exponential in the Na^+-chloroplasts. According to Delosme [18] the sigmoidal shape characterizes an excitation-transfer between PS II connected units; otherwise the induction is exponential. The results shown in fig.3 means that when the spill-over increases (+Na) there is a decrease in the yield of the energy-transfer between System II units; the value of this transfer is restored by the addition of Mg^{2+}.

A second argument which indicates that the spill-over is low in O-salt system has been given by Wydrzynski et al. [2]. They showed that in sucrose washed chloroplasts the ratio of PS II over PS I fluorescence emissions at 77°K is higher, as in ($NaCl + MgCl_2$) chloroplast, than in NaCl plastids.

These data which show the same pattern in 2 ionic conditions: O-salt and ($Na^+ + Mg^{2+}$) and which point out the antagonistic effect at low concentrations of Na^+ and Mg^{2+} are in agreement with Barber and Mills [19] explanation on salt effect on fluorescence, i.e., they are not produced by a change of the ionic strength, nor by binding of specific cations to the membrane but they may be monitored by electrostatic interaction in the chloroplastic membrane according to the 'Diffuse double-layer theory'.*

The τ–o curves of fig.2 show a slight but significant difference in the slope between the Na^+-chloroplast on the one hand and the *O*-salt and ($Na^+ + Mg^{2+}$) samples on the other hand. Such a difference between Na^+-chloroplast and ($Na^+ + Mg^{2+}$) chloroplast was not obvious in a previous experiment [6] done without an averager device.

If the relaxation of PS II excitons is an exponential decay then:

$$\tau = \frac{\tau_o}{a \cdot I} . F$$

where F = fluorescence intensity, τ_0 = intrinsic lifetime of fluorescence, I = incident light intensity absorbed and a = the fraction of this intensity absorbed by PS II. In the absence of any change of the total light absorbed (absorption spectrum) depending upon salt conditions, a change of the τ–$\Phi(F)$ slope will correspond to a variation of a. Consequently we must conclude that in sucrose washed and ($Na^+ + Mg^{2+}$) sample the sensitization of PS II is slightly larger than in Na^+ chloroplasts. This result confirms the results of Butler, Kitajima and Loos [8,9]. During the time course of the induction without DCMU there are weak transitory variations of the slope; especially, there is a discontinuity of the τ–Φ slope at the end of the initial $O \rightarrow I$ phase of the fluorescence rise. According to the previous statements this will correspond to a variation of a. This may be related to the changes of $F < 690$ nm/$F > 715$ nm ratio observed by Schreiber and Vidaver [20] in their fluorescence induction curves.

* In this picture, fluorescence changes reflect the total positive diffusable charge immediately adjacent to the membrane

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