*Biochimica et Biophysica Acta*, 592 (1980) 296—302

BBA 47905

# REGULATION OF EXCITATION ENERGY DISTRIBUTION IN PHOTOSYSTEM-II FRAGMENTS BY MAGNESIUM IONS *

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(Received December 13th, 1979)

*Key words: Photosystem II; Energy transfer; Fluorescence quenching; Light-harvesting complex; Cation effect; (Regulation, subchloroplast)*

## Summary

Fluorescence characteristics of Photosystem-II subchloroplasts (TSF-II and TSF-IIa) fractionated by Triton X-100 treatment were studied in relation to cation-induced regulation of excitation-energy distribution within subchloroplast fragments. Absorption spectra and fluorescence-emission spectra at 77 K showed that TSF-II contains the light-harvesting chlorophyll-protein complex in addition to the reaction-center complex, which is present alone in TSF-IIa.

$Mg^{2+}$ increased the ratio of $F_{695\mathrm{nm}}$ to $F_{685\mathrm{nm}}$ in the fluorescence-emission spectrum of TSF-II particles at 77 K, but had no effect on TSF-IIa particles. $Mg^{2+}$ also induced a quenching of chlorophyll fluorescence at room temperature in TSF-II, an effect that was insensitive to the presence of DCMU. The DCMU-insensitive fluorescence quenching was not observed in the TSF-IIa preparation. These results suggest an existence of cation-induced regulation of excitation-energy transfer in TSF-II preparations. Presence of antenna chlorophyll molecules alone does not seem to be sufficient for observing energy-transfer regulation by cations in Photosystem-II preparations.

## Introduction

Cations regulate the distribution of excitation energy between Photosystem I and Photosystem II in chloroplasts [1,2], and a possible site of regulation has been shown to be on a light-harvesting chlorophyll-protein complex in the

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* Contribution No. 681 from the Charles F. Kettering Research Laboratory.

Abbreviations: TSF-II (TSF-IIa), Triton-fractionated Photosystem-II subchloroplast fragments (subfragments produced by further fractionation of TSF-II); DCIP, 2,6-dichlorophenolindophenol; DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea; Q, primary electron acceptor of Photosystem II; SDS, sodium dodecyl sulfate; Chl; chlorophyll.

thylakoid membrane [3]. Cations bound to the light-harvesting complex may bring about some conformational changes in the microenvironment of the regulation site and enable efficient control of energy distribution [4].

Although both photosystems are required to observe the effects of cations on energy transfer in chloroplasts [5,6], some authors have shown the presence of cation-induced regulation of excitation-energy distribution in subchloroplast particles [7—12]. In the accompanying paper [13], we showed that $Mg^{2+}$ regulates electron transport, but not excitation-energy distribution in the Photosystem-II reaction-center particles (TSF-IIa) fractionated from spinach chloroplasts by Triton X-100 treatment.

The TSF-IIa particles were shown to contain no light-harvesting complex, as inferred from its polypeptide pattern in SDS gel-electrophoresis; the 23 000-dalton polypeptide component that has been ascribed to the light-harvesting chlorophyll-protein complex [14,15] was significantly diminished compared to the TSF-II fraction, the starting material from which TSF-IIa was isolated [16]. Furthermore, the TSF-IIa particles have very small amount of chlorophyll *b* [17], which is an essential component of the light-harvesting chlorophyll-protein complex [18—21].

In this paper, we distinguish the two Photosystem-II preparations, TSF-II and TSF-IIa, with respect to their chlorophyll-fluorescence characteristics, and show evidence for the presence of energy-transfer control by $Mg^{2+}$ in the TSF-II fraction that contains a light-harvesting chlorophyll-protein complex in addition to the Photosystem-II reaction-center (core) complex.

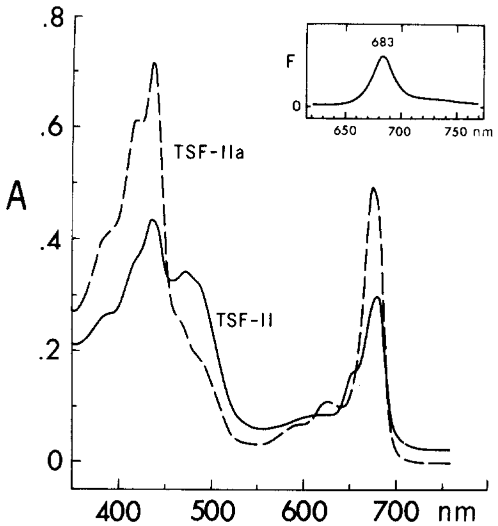
## Materials and Methods

Preparation and characteristics of the subchloroplast fragments (TSF-II and TSF-IIa) and procedures for absorption-change and fluorescence measurements have been described in the accompanying paper [13]. When adding reagents to the illuminated cuvette during fluorescence measurements at room temperature, the reaction mixture was stirred momentarily after the addition. Other conditions are described in the figure legends.

## Results and Discussion

Photosystem-II reaction-center particles, TSF-IIa, were isolated by further purification of the TSF-II fraction. These particles showed enriched activity of *P*-680 and *C*-550, and a high chlorophyll *a*/*b* ratio (28 ± 2) [17]. A large decrease in chlorophyll *b* in TSF-IIa particles compared to TSF-II is shown in the absorption spectra in Fig. 1. TSF-IIa has its absorption maximum at a shorter wavelength in the red region and there is no shoulder at 650 nm. It was previously reported that there was a decrease in the amount of both chlorophyll *b* and xanthophylls in TSF-IIa particles [18]. Fluorescence-emission spectra of TSF-II and TSF-IIa at room temperature show a peak at 680—685 nm as reported previously by Ke et al. [19] (Fig. 1, inset).

Fig. 2 shows fluorescence-emission spectra of TSF-II and TSF-IIa at 77 K. In TSF-IIa, there is a single peak at 687 nm and a small shoulder in the 720—750 nm region. On the other hand, TSF-II fragments show a peak at 691 nm and



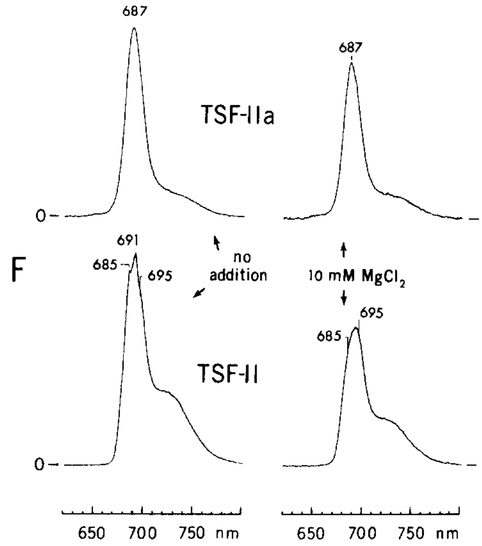


Fig. 1. Absorption spectra of TSF-II and IIa preparations at room temperature. The TSF-II spectrum was displaced vertically by 0.05 absorbance units for clarity. The room-temperature fluorescence-emission spectrum of TSF-II is shown in the inset. The emission maximum (683 nm) is placed at the correct location on the wavelength scale of the absorption spectrum, but the emission spectrum has a different wavelength scale.

Fig. 2. Fluorescence emission spectra of TSF-II and IIa in the presence and absence of $MgCl_2$ at liquid nitrogen temperature. Concentrations of chlorophyll in the preparations were 15 µg Chl/ml in TSF-IIa and 35 µg Chl/ml in TSF-II, respectively. $MgCl_2$ (10 mM) was added in the dark before freezing the preparation. The data are not normalized on the chlorophyll basis.

shoulders at 685 nm, 695 nm and in the 720—750 nm region. The shoulders at 685 nm and 695 nm were sometimes obscured depending on the condition of the preparation. Contribution of the 720—750 nm emission was relatively enhanced in the TSF-II preparation, and that may be, to a very small extent, due to some contamination of Photosystem-I antenna chlorophylls in the fraction. Although no definite information is yet available about fluorescence characteristics of the light-harvesting chlorophyll-protein complex, the fluorescence emission spectrum of TSF-II at low temperature shows presence of light-harvesting complex, which is reported to have one fluorescence emission peak at 685 nm [20,21]. Satoh and Butler [22] fractionated subchloroplast fragments corresponding to Photosystem I, Photosystem II and light-harvesting chlorophyll *a*/*b* protein by digitonin treatment of chloroplasts, followed by sucrose-density-gradient centrifugation, DEAE-cellulose chromatography and electrofocusing. Their light-harvesting complex showed fluorescence-emission spectra with a maximum at 681 nm at 77 K. Thornber et al. [21] reported that treatment of the isolated light-harvesting complex with Triton X-100 substantially shifted the red and Soret maximum of chlorophyll *b* to lower and higher energies, respectively. Thus, in Triton X-100 treated subchloroplast fragments some changes may occur in the characteristics of the absorption and fluorescence spectra.

Effects of $Mg^{2+}$ were examined on low-temperature fluorescence-emission spectra of Photosystem-II preparations (Fig. 2). In TSF-IIa, there was no significant change in the spectrum by the addition of $MgCl_2$ at the concentration range from 1 mM to 10 mM. The same concentration of KCl also had no effect on the emission spectra. On the other hand, $MgCl_2$ brought about a small but definitely discernible change in the emission spectrum of TSF-II preparation at 77 K; in the presence of $MgCl_2$, the emission peak was shifted to longer wavelength by 2—3 nm and the height of the 685 nm shoulder decreased correspondingly. $Mg^{2+}$ had no effect on the absorption spectrum of TSF-II or IIa preparations (data not shown). The effect of $Mg^{2+}$ was dependent on the conditions of TSF-II; no shift of fluorescence peak position was observed by the addition of $MgCl_2$ in preparations aged for several hours at 4° C.

When we examined the ratio of $F_{695nm}$ to $F_{685nm}$ in both Photosystem-II preparations in the presence and in the absence of cations, there is an increase in the $F_{695nm}/F_{685nm}$ ratio of TSF-II by the addition of $MgCl_2$, but that is not the case in TSF-IIa particles (Table I). $MgCl_2$ induced a quenching of chlorophyll fluorescence in TSF-II preparation at room temperature (Fig. 3 and Table II). The quenching was independent of the presence of DCMU. $CaCl_2$ induced a quenching of fluorescence but to a lesser extent (data not shown). The quenching effect of $MgCl_2$ was saturated at 5 mM using TSF-II at 17.5 $\mu$g Chl/ml. KCl had no effect on chlorophyll fluorescence at the same concentration level. On the other hand, $Mg^{2+}$-induced quenching of fluorescence in TSF-IIa particle was highly sensitive to DCMU; a small or no quenching of fluorescence was induced when TSF-IIa was preincubated with 5 $\mu$M DCMU. As suggested in the accompanying paper [13], $Mg^{2+}$-induced fluorescence quenching in TSF-IIa reflects an accelerated oxidation of Q in the presence of $MgCl_2$, and not attributable to cation-induced regulation of excitation-energy distribution in the particles.

Our results show the possibility that in TSF-II preparations which contain a light-harvesting chlorophyll-protein complex in addition to the Photosystem-II reaction-center complex, there is a cation-induced control of energy transfer between the light-harvesting complex and the reaction-center complex. Gross and co-workers previously showed $Ca^{2+}$-induced quenching of chlorophyll fluorescence in a TSF-II preparation [10] and cation-induced decrease in quantum yield of Photosystem-II reactions monitored by diphenyl carbazide-dependent DCIP-photoreduction [11]. The 77 K fluorescence-emission spectra of their Photosystem-II preparations showed a peak at 685 nm and a shoulder at 725 nm, but no shoulder at 695 nm which would correspond to the Photosystem-II

TABLE I

EFFECT OF $MgCl_2$ ON THE $F_{695}/F_{685}$ RATIO IN TSF-IIa AND TSF-II PARTICLES AT 77 K

Chl concentrations: 15 $\mu$g Chl/ml for TSF-IIa and 35 $\mu$g Chl/ml for TSF-II.

| $MgCl_2$ | TSF-IIa | TSF-II |
|---|---|---|
| none | 0.76 | 0.91 |
| 1 mM | 0.76 | 1.16 |
| 10 mM | 0.75 | 1.24 |

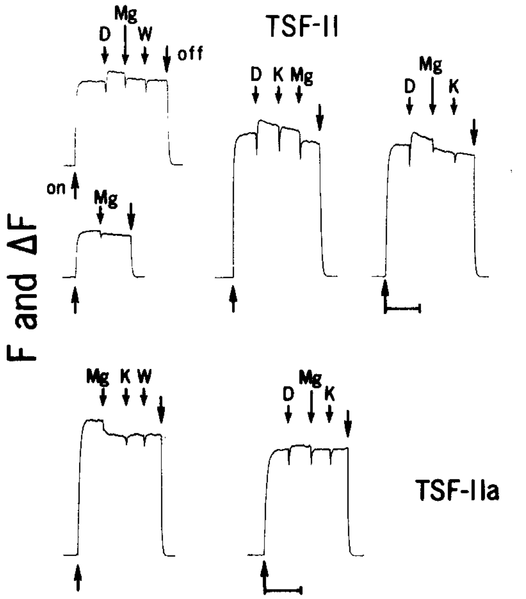
TABLE II

EXTENT OF CHLOROPHYLL-FLUORESCENCE QUENCHING BY CATIONS

TSF-IIa and TSF-II both at 17.5 μg Chl/ml; DCMU concentration, 5 μM; all salt concentrations, 5 mM.

| | Conditions | % Quenching of fluorescence | |
|---|---|---|---|
| TSF-IIa | −DCMU | KCl | 1.0 |
| | | $MgCl_2$ | 12.0 |
| | +DCMU | KCl | 0 |
| | | $MgCl_2$ | 1.3 |
| TSF-II | −DCMU | KCl | 0 |
| | | $MgCl_2$ | 7.0 |
| | +DCMU | KCl | 0 |
| | | $MgCl_2$ | 8.0 |
| | | $CaCl_2$ | 4.2 |

trap. They noted a decrease in the amplitude of the 685 nm band by the addition of cations, but did not observe a change in the $F_{695nm}$ to $F_{685nm}$ ratio that is an indicator for the control of energy distribution from a light-harvesting complex to the reaction-center complex. From these results they concluded that in the Photosystem-II preparations, cations create quenching centers in



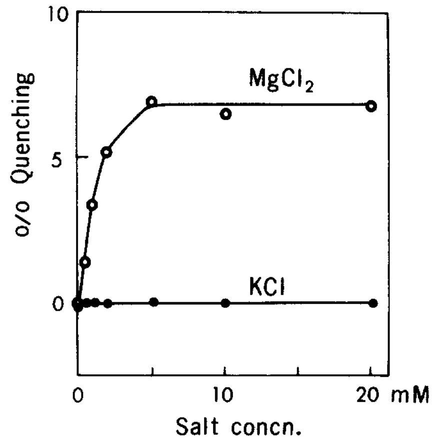


Fig. 3. $MgCl_2$-induced quenching of chlorophyll fluorescence in Photosystem-II preparations. Concentration of chlorophyll in both TSF-II and IIa was 17.5 μg/ml. $MgCl_2$ (Mg) or KCl (K) were 5 mM, and 5 μM DCMU (D) was added where indicated. W represents 10 μl distilled water. Amplitude of fluorescence is not normalized on the chlorophyll basis. Upward and downward (larger) arrows represent excitation light on and off. The two bars represent 100 s.

Fig. 4. Dependence of chlorophyll fluorescence quenching in TSF-II preparation on the concentration of cations. The TSF-II sample contained 17.5 μg Chl/ml and 5 μM DCMU.

the Photosystem-II antenna pigments which serve to regulate Photosystem-II activity. However, in our TSF-II preparations, there was a 695 nm shoulder in addition to a 685 nm shoulder in the low-temperature fluorescence spectra, and the ratio of $F_{695\mathrm{nm}}$ to $F_{685\mathrm{nm}}$ was markedly changed by the addition of $MgCl_2$. We interpret these results as an enhancement of energy transfer from the light-harvesting complex to the Photosystem-II trap. Energy transfer from a light-harvesting complex to the reaction-center complex in Photosystem-II preparations may increase the quantum efficiency of Photosystem-II reactions. The quantum yield of Photosystem-II reactions cannot be easily determined from the measurement of DCIP photoreduction using diphenyl carbazide as an electron donor, because there is a cation-dependent regulation site of electron transport on the oxidizing side of Photosystem II, located probably between diphenyl carbazide and *P*-680 [13]. We observed an accelerated cyclic electron flow around Photosystem II in the presence of $MgCl_2$, although the diphenyl carbazide-supported DCIP photoreduction was inhibited under the same conditions. The accelerated cyclic electron transport was observed even in TSF-IIa preparations where no regulation of energy distribution exists, suggesting that the effect of $Mg^{2+}$ on the electron-transport rate is independent of the cation-induced change in quantum efficiency of the light reaction.

Thus, there are various effects of cations on the thylakoid membrane of Photosystem-II preparations, including energy transfer regulation [7—12], electron transport [13], membrane conformational change [23], and some rather non-specific electrical regulations of photosynthetic reactions [24]. Present experimental results were obtained under conditions where a high buffer concentration (20 mM sodium phosphate, pH 7.0) was used, and the observed effect of $Mg^{2+}$ can definitely be differentiated from changes of membrane surface potential by the addition of cations (further details on the effects of cations on the surface electric properties in subchloroplast fragments will be reported elsewhere).

Appearance of regulations may be dependent on molecular composition and architecture of the membrane, as well as the surrounding ionic environment. The most significant difference between TSF-II and TSF-IIa preparations is the presence of a light-harvesting pigment-protein complex in TSF-II. Presence of the light-harvesting complex appears to induce cation-dependent regulation of excitation-energy transfer even in subchloroplast preparations. The presence of antenna chlorophylls of Photosystem II are probably not sufficient to obtain regulation of energy transfer.

## Acknowledgement

This work was supported in part by a grant (PCM-8003702) from the National Science Foundation.

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