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THE THERMAL AND THE ION EFFECTS ON THE LOW TEMPERATURE EMISSION SPECTRUM OF SPINACH CHLOROPLAST[1],[2]

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Abstract

The thermal and the ion effects on the low temperature (77°K) emission spectrum of spinach chloroplast were studied. It is found that, 1), F695[4] band persists in spinach chloroplasts with thermal inactivated H_2O splitting system, higher temperature reduces F695 band to a shoulder of F685; 2), the value of F695/685 is influenced by exogenous chloride ion, so is the system II photochemistry; 3), the value of F735/F685 is changed by the introduction of sodium ion, thermal effects on this value depend on the presence of salts. Our observations suggest that the photosystem II reaction center may function as a "sensitizer" not engaged in a redox reaction in the electron transfers. They also suggest that one of the sites of chloride effects is at the photosystem II reaction center or its vicinity.

Introduction

Three conspicuous fluorescence emission bands of chloroplast exist at 77°K; they are F685, F695 and F735[4]. On the basis of excitation spectra for fluorescence (Bergeron and Olson, 1964; Murata *et al.*, 1966; Govindjee and Yang, 1966; Goedheer, 1968; Cho and Govindjee, 1970) and of emission spectra of fractionated subchloroplast particles (Boardman *et al.*, 1966; Ke and Vernon 1967), F685 and F695 can be assigned to photosystem II, and F735 to photosystem I. The chlorophylls emitting F695 band are sensitive to the addition of polar molecules (Cho and Govindjee, 1970) and to a change in the phase of ice (Govindjee and Yang, 1966; Krey and Govindjee, 1966; Cho and Govindjee,

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(4) F685, F695, F735 denote fluorescence bands with peak wave-lengthes at about 685 nm, 695 nm, 735 nm. F693, F695 and F698 are assumed to be the same band in different organisms, so are the F723 and F735.

1970). The emitters of F695 seem to situate in an aqueous environment where chlorophylls and enzymes interact. This property, together with the results of fluorescence induction analysis at low temperatures (Kok, 1963; Murata, 1968; Donze and Duysens, 1969) suggest that the F695 originates from the reaction center of photosystem II.

Various chemicals can alter the low temperature emission spectrum. Among them, the hydroxylamine lowers F695 selectively when cells of P. cruentum have been preilluminated with white light (Mohanty *et al.*, 1971); dimethylsulfoxide improves the resolution of F695 band and decreases F723 in *C. pyrenoidosa* (Cho and Govindjee, 1970); divalent cations enhance F685 and F695 while reduce F730 in spinach chloroplasts (Murata, 1969); 8 M urea depresses F685 and F695 dramatically while 1 mM o-phenanthroline in the presence of 1 M urea increases F695 remarkably in spinach chloplasts (Satoh, 1972); *Ricinus* leaf extract is found to induce complicate changes in the emission spectrum of chloroplasts (Nathanson and Brody, 1970). Heath and Hind (1969) have shown an enhancement of F693 in the presence of chloride and attribute its effect to a shift in the redox state of the photosystem II reaction center prior to freezing.

By studying the differential thermal and salt effects on photo-system II, it is found that a functional H_2O splitting system is not essential for the emission of F695, which indicates that the chloride effect may not be due to an altered redox poise of Q. A striking change of the value of F735/F685 in the presence of 10 mN of Na^+, but not in the presence of 10 mN of Ca^{++} is also reported.

Material and Method

Spinach (Spinacia oleracea) from market was hand ground in a medium consisting of 50 mM tricine and 150 mM sucrose (pH 7.3). After removal of cell debris by straining through muslin, chloroplasts were spun down with a table top centrifuge (International equipment Co. International Clinical centrifuge Model CL) runing at its top speed for one minute. Pellet was resuspended in distilled H_2O and used immediately.

Low temperature fluorescence emission were measured at an angle 90° from the exciting beam, a Bausch and Lomb grating monochromator (500 mm focal length, f4.5), plus red filters, was used for spectrum analyzing. Mechanically modulated blue light isolated with glass filters excited the chloroplasts which were held in a glass capillary with about 2 mm inner diameter. The capillary was dipped in a Dewar containing liquid N_2. Signals were fed into a "lock-in amplifier" (Princeton Applied Research, Model HR-8). The spectra were corrected for the spectral efficiency of the photomultiplier and the transmittance of filters, but not for the efficiency of monochromator, and then normalized at

685 nm. Reabsorption above 685 nm was not serious under our conditions (Li, 1974).

Photoreduction of $NADP^+$ was followed on a Model 14 Cary recording spectrophotometor (Gorman and Levine, 1965).

Result and Discussion

Table 1 shows a four-fold increase in DCIP reduction rate upon the addition of 10 mM sodium chloride or 5 mM calcium chloride to the unbuffered chloroplast suspension containing no exogenous salt. Chloride is also essential for a better resolving of F695 in low temperature emission spectrum (fig. 1), potassium iodide has no effect on F695 (data not shown). Heating of the

Table 1. *Chloride Effect on the DCIP Reduction*

Reaction was followed on a Model 14 Cary recording spectrophotometer. The cuvette in the sample compartment contained chloroplasts. 5 μg chl/ml. DCIP, 0.05 mM and other additions as shown in the table. The DCIP was omitted from the cuvette in the reference compartment.

Addition	Conc. (mM)	DCIP reduced (μ moles/mg chl·hr)
No addition		25
NaCl	10	90
$CaCl_2$	5	115

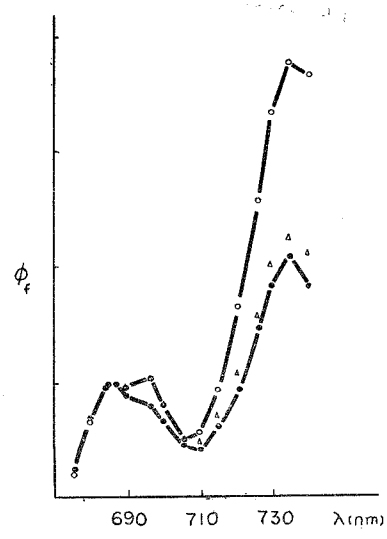

Fig. 1. Effects of Ions on the Fluorescence Emission Spectrum at 77°K (spectra normalized at 685 nm). Chloroplasts were suspended in distilled H_2O (5 μgchl/ml) and cooled to 77°K rapidly. ●: No addition; △: $CaCl_2$, 5 mmM; ○: NaCl, 10 mM; ϕ_f: relative fluorescence yield.

chloroplasts at 65°C for five minutes reduces the F695 band (Goedheer, 1968) to a shoulder of the F685 band (fig. 2). The same chloroplast preparation has a nil $NADP^+$ reduction rate with photosystem II electron donor (table 2) which indicates inactivation of the photosystem II reaction center. Under a lower temperature which inactivates the H_2O splitting system (Katoh and San Pietro, 1967; Böhme and Trebst, 1969) no deleterious effects are observed on

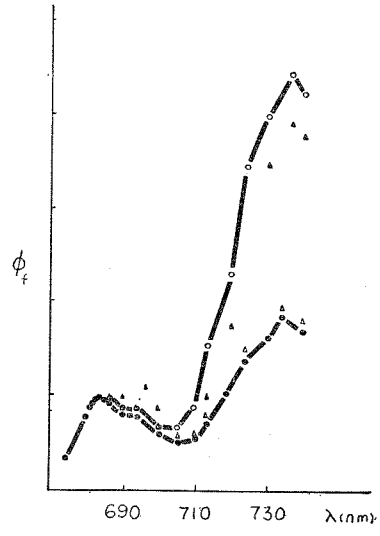

Fig. 2. Effects of Heating on the Emission Spectrum at 77°K (spectra normalized at 685 nm). Diluted chloroplast suspension (5 μgchl/ml) was heated at 65°C for 5 minutes in the absence (●) or presence of 10 mM NaCl (○). NaCl added after heating (△) shows no effect on F735. ▲: Chloroplasts without heating; ϕ_f: relative fluorescence yield.

Table 2. *Thermal Effects on the Photochemistry of Photosystems I and II*

Reaction medium contained: chlorplasts, 5 μg chl/ml; NaCl, 10 mM; NADP, 0.25 mM; saturated amount of ferredoxin and ferredoxin-NADP reductase; photosystem I or II donor couples. System I donor couple: DCIP, 0.05 mM, ascorbate (pH 7.0) 5 mM. DCMU, 5×10^{-5} M, added together with system I sonor. System II donor couples: 200 μM hydroquinone; 300 μM ascorbate. Ferredoxin, ferredoxin-NADP reductase and NADP were omitted in the cuvette in the reference compartment. Chloroplasts in the presence of NaCl were heated separately.

Treatment	Donor couples	NADP reduced μ moles/mgchl hr
No heating	system I	28
	system II	74
Heated chloroplasts 65°C, 5 minutes	system I	40
	system II	0

the F695 band nor on the photosystem II donor reduction. Again, both the F695 and the photochemistry are chloride-dependent. (data not shown). The results indicate that an intimate relationship exists between a functional photosystem II reaction center and the F695. The dependence of F695 on chloride and its independence on a functional H_2O splitting system is an observation contradicted to the proposed site of action of chloride based on photosystem II electron donor experiments reported by Izawa *et al.* (1969). They found photosystem II electron donor reactions were independent of chloride and concluded that the latter acts at a site close to the H_2O splitting system. This discrepency in chloride effect may be due to differences in the states of chloroplasts employed in the two experimental systems, or it may be due to the fact that chloride is not an indispensable cofactor for the photosystem II reaction center, the electron donors ascorbate or hydroxylamine at the concentrations used by Izawa *et al.* may substitute for the role played by chloride under our experimental condition.

Döring and co-workers (Döring *et al.*, 1967; Döring *et al.*, 1969; Döring, 1970; Govindjee *et al.*, 1970) report an absorbance change due to the active chlorophyll a in system II with peaks at 682 nm (in system II particles) and at 690 nm (P690 in chloroplasts). Cho and Govindjee (1970) suggest that absorbance change at 690 and F698 arise from related components. Later, Döring *et al.* (1969) suggest that the net electron transport is not necessary for observing the absorbance change of P690. They further postulate that P690 acts as a "sensitizer" not engaged in a redox reaction in the electron transfers. An unknown energy acceptor may transfer this excitation energy into electronic energy (Döring *et al.*, 1967; Döring *et al.*, 1969; Döring, 1970; Govindjee *et al.*, 1970). Our observation that F695 persists in the absence of a functional H_2O splitting system may support their suggestion.

While both NaCl (not KI) and $CaCl_2$ raise the value of F695/F685, only NaCl changes the value of F735/F685 (fig. 1). The increase of F735/F685 is not affected by the heat-treatment which abolishes F695 (65°C, 5 minutes in the presence of NaCl). Heating in the absence of exogenous ions has no effect on the value of F735/F685. Addition of NaCl after heating does not change the ratio (fig. 1). The effects of sodium and other agents on fluorescence may provide a clue for the study of the different constitutents or environments of the two types of the reaction centers.

In summary, data in this report have shown that a functioning H_2O splitting system is not essential for the existing of F695 which implies that the reaction center chlorophyll may not be an electron carrier but an "energy sensitizer". The value of F695/F685 influenced by the presence of exogenous chloride, while the value of F735/F685 is changed by the introducing of 10 mM sodium ion.

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熱和離子對菠菜葉綠體低溫放射光譜的影響

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葉綠體在77°K時，其螢光放射光譜顯示三個尖峯，三者因其波長而定名爲 F685，F695 及 F735。本文報導加熱與離子對這三種螢光的影響，茲將其效果分列如下：(一)五分鐘的熱處理(60°C)能抑制 F695 的强度，較低溫度(50°C，5分鐘)的處理，可使水分解系統遭破壞，但却不影響 F695；(二)室溫時的第二光系統化學反應與低溫時 F695/F685 比值同受外加氯離子的影響；(三)鈉離子改變 F735/F685 的值，熱處理亦影響 F735，但需在具有外加離子的狀況下。

以上的觀察指出：(一)第二光系統的反應中心可能僅是一「能量感受中心」(Energy sensitizer)，而不是電子載體；(二)氯離子的作用點之一可能是在第二反應中心附近。