THE EFFECT OF SODIUM IONS ON THE LOW TEMPERATURE FLUORESCENCE SPECTRA AND ULTRASTRUCTURE OF THE GRANUM MEMBRANE

By

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In chloroplasts, in isolated grana and in fragments of stroma lamellae the effect of Na^+ ions was studied on the low temperature fluorescence spectra and on the ultrastructural pattern of chloroplast lamellae.

The data have shown a complex relationship between the salt-induced change of integral fluorescence and lamellar appression, indicating at least two competing effects. The increase of the F_{695}/F_{685} ratio observed in chloroplasts and grana may be reflecting the alteration of the molecular structure of Photosystem 2, while the increase of the F_{715}/F_{735} ratio can be connected with subultrastructural rearrangements of Photosystem 1.

Introduction

Optimum efficiency of photosynthesis requires a balanced input of quanta to both photosystems. Partitioning of quanta is controlled in general by the relative cross-sections of the photosystems, but in higher plant chloroplasts, also a second mechanism is incorporated which regulates the distribution of quanta. This mechanism operated by the light-harvesting pigment protein is largely influenced by meta cations. As a result, small amounts of divalent and large amounts of monovalent cations can affect considerably the distribution of excitation energy, thus inducing characteristic changes in the fluorescence spectra of chloroplast membranes [1—3]. The cation-regulation of excitation energy distribution is accompanied by ultrastructural changes which lead to the fusion of adjacent membranes forming the lamellar stacks of grana [4]. This series of events correlate the chloroplast ultrastructure with the fluorescence characteristics, and has been the subject of many publications.

These works demonstrated the coincidence of the cation-induced fluorescence and ultrastructural changes, but they could not establish to what extent the process of lamellar appression contributes to the cation regulation of quantum distribution [5].

In this paper we have studied the correlation between the ultrastructural pattern and the low temperature fluorescence spectra of chloroplasts treated with Na^+-ions in a wide concentration range.

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Materials and methods

Seedlings of maize (Zea mays L cv. MV 651) were grown in the greenhouse for 9—12 days. Chloroplasts were isolated from the mesophyll of the first leaves in a medium containing 0.35 M sucrose and 0.05 M TRIS, pH 7.5. Grana and stroma lamellae were separated by ultrasonication and differential centrifugation [6].

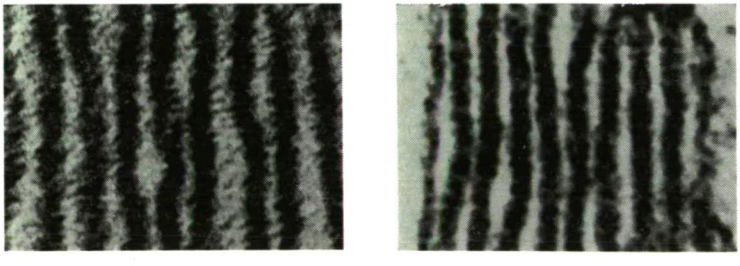
Isolated chloroplasts, grana or stroma lamellae were suspended in media containing 0.35 M sucrose and various concentrations of NaCl (0.0, 0.1, 0.4 and 1.0 M, respectively). The suspensions were adjusted to 10^{-5} M chlorophyll content and were incubated at 0° for 10 min in the dark.

For electron microscopy the suspensions were centrifuged at 30 000xg for 45 min. The pellets were fixed with 0.1 M glutaraldehyde, postfixed with OsO_4 and embedded in Araldite. Ultrathin sections were prepared on a Porter-Blum ultramicrotome and stained with lead citrate. Electron micrographs were obtained at a magnification of 20 000 in a JEOL 100/B electron microscope. Thickness of the membranes was recorded by microdensitometry on the electron microscope negatives [7].

Fluorescence measurements were performed with dilute suspensions in liquid N_2 with a cell thickness of 0.2 mm [8]. The excitation wavelength was set at 435 nm (band width 5 nm). Emission spectra were detected with a slit adjusted to 4 nm by an RCA 31 034/a multiplier and were corrected for the response of the emmission monochromator and photomultiplier.

Results and discussion

High resolution electron microscopy revealed that granum thylakoids were stacked even in the absence of Na^+-ions. The salt-effect could be seen on the thickness of the double membrane of grana (Fig. 1.). These decreased with increasing concentration of NaCl (Table 1). In the chloroplasts depleted of ions the thickness of double membranes was about the same as for two single membranes. This suggests that in the absence of ions the double membranes slide apart and stacking is due only to a surface contact. With increasing concentration of salt a process of mutual interlocking of the adjacent membranes occurred. The thickness of double membranes measured in chloroplasts in situ [7] corresponded to that of the membrane thickness obtained with 0.4 molar salt. Thus we can suppose that the mutual interlocking of membranes



A *B*

Fig. 1. Changes in the thickness of granum thylakoids. *A*: granum membranes in sucrose containing no salt, *B*: granum membranes in sucrose containing 1 M salt (250 000x)

Table I

The effect of Na^+-ions on the membrane thickness of granum and stroma thylakoids (average and standard deviation from 25 thylakoids)

Material	Concentration of NaCl (M)			
	0.0	0.1	0.4	1.0
Granum membrane (Å)	153±17	115±10	108±9	102±9
salf effect %		−25	−29	−33
Stroma membrane (Å)	68±8	67±8	69±7	68±7
salt effect %		(−1)	(+1)	(0)

values in parentheses: not significant

does exist also *in vivo*. In the literature, one can encounter such data which show that with very low doses of Na^+-ions, the membranes separated [9]. We did not observe this phenomenon with any of the salt solutions applied. With single membranes of stroma lamellae salt dependent changes could not be detected in the membrane thickness.

A representative set of the low temperature fluorescence spectra is shown on the Fig. 2.

In general terms the lowest fluorescence yield was found under the same conditions where the thickness of the membranes was the same as in chloroplasts in situ — that is in the presence of 0.1—0.4 M NaCl.

In addition to the salt effect on the integral fluorescence intensity, variations of the spectral distribution of fluorescence were also observed (Fig. 3). The main fluorescence bands with maxima at 685 and 735 nm show the same trend as the integral fluorescence intensity although to a different extent. The intensities emitted at 695 and 705 nm are more or less constant. The change of the fluorescence at 715 nm (representing a hidden band at around 720 nm) deviates from the formers since it shows a monotonous increase as a function of the Na^+-ion content. The small dip can be accounted for the overlap by the strong band at 735 nm.

The cation-induced fluorescence chan-

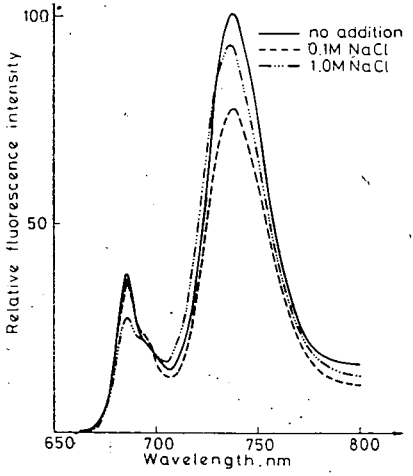


Fig. 2. Low temperature (77 °K) fluorescence emission spectra of chloroplasts in a sucrose medium containing no salts (no addition) or after a 10 minute incubation with 0.1 or 1.0 M NaCl (For further details see Materials and methods)

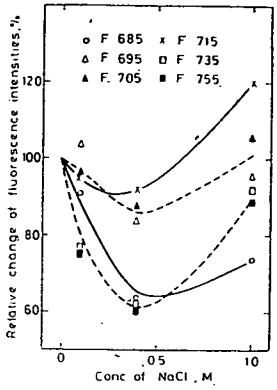


Fig. 3. Changes in low temperature (77 °K) fluorescence intensities of chloroplasts as a function of NaCl concentration

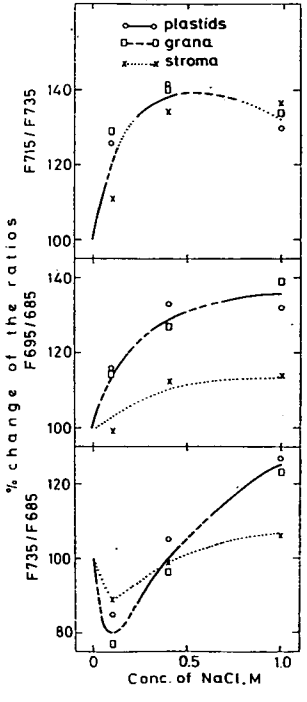


Fig. 4. Changes of the ratios of low temperature (77 °K) fluorescence intensities of chloroplasts and fragments

ges at various wavelengths can be conveniently characterized by the ratios of fluorescence intensities (Fig. 4). The ratios of fluorescence intensities at characteristic wavelengths were compared in the spectra of whole chloroplasts, isolated grana and stroma membranes.

$F\,715/F\,735$ increased as a result of Na^+ treatment not only with chloroplasts and grana but also with stroma membranes. The same extent of the change with different types of preparations excludes that the response of the stroma membranes was to be attributed to contamination. This cation-induced fluorescence change is peculiar in the sense that it occurs not only in stacked membranes (containing both photosystems) but also in single lamellae which have only Photosystem 1. Since the thickness of stroma membranes was not affected by salt-treatment we can conclude that the alteration of the fluorescence at 715 nm cannot be connected with gross structural changes of the thylakoid membranes, but rather with a subultrastructural rearrangement of Photosystem 1.

The cation induced change of the $F\,695/F\,685$ ratios was an increase as a function of the concentration of Na^+-ions. This change was about the same with chloroplasts and isolated grana and very slight with the stroma membranes. It can be suggested that this change reflects the alteration of the molecular architecture of Photosystem 2, and can be correlated with ultrastructural change.

The cation induced change of the $F\,735/F\,695$ ratio with chloroplasts and grana at low concentrations was similar to the change reported by WYDRZYNSKY *et al.* [10]. At higher salt concentrations however the tendency of the change was reversed. The reversal of the change shows the action of two competing effects which cannot be correlated with the monotonous decrease of the thylakoid thickness. The complexity of effects and diverse views in the literature suggest that more than one mechanism is involved in the action of salts [11, 12]. With stroma membranes the change was much slighter. In this case as well as in the case of the slight change observed in the $F\,695/F\,685$ ratio in stroma membranes one can suggest that the changes are due to contamination by granum fragments.

Our data have shown that some cation-induced

fluorescence changes are intimately connected with the membrane thickness some fluorescence changes are not.

The relationship between lamellar appression and fluorescence proved to be complex and indicated at least two competing effects.

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ВЛИЯНИЕ ИОНОВ НАТРИЯ НА НИЗКОТЕМПЕРАТУРНУЮ ФЛУОРЕСЦЕНЦИЮ И УЛЬТРАСТРУКТУРУ МЕМБРАН ГРАН

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Изучали влияние ионов натрии на низкотемпературную флуоресценцию и ультраструктуру хлоропластов, изолированных гран и фрагментов ламеллы стромы.

Данные показывают сложную связь между индуцированным солью изменением интегральной флуоресценции и ламелярным слиянием, указывающую наличием — по крайнем мере — двух конкурирующих процессов. Увеличение отношения Φ_{695}/Φ_{685} обнаруженного в хлоропластах и гранах возможно отражает изменения молекулярной структуры фотосистемы 2, в то время как увеличение отношения Φ_{715}/Φ_{735} можно связать с перестройкой фотосистемы 1 на молекулярном уровне.