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# LOW TEMPERATURE FLUORESCENCE SPECTRA OF THE WILD TYPE AND OF A CHLOROPLAST MEMBRANE MUTANT OF CHLAMYDOMONAS REINHARDI. THE RELATIONSHIP BETWEEN FLUORESCENCE REABSORPTION AND MEMBRANE STACKING[1]

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**Abstract**

Enhanced ratio of F715/F687 at 77°K is found in chloroplast membrane mutant ac-5 cells of *Chlamydomonas reinhardi*. The enhancement is not correlated with the degree of stacking of the thylakoid membranes, for the unstacking mixotrophic ac-5 cells have an F715/F687 ratio between those of wild type and phototrophic ac-5 mutant, both of them have stacking membranes. The states of membrane stacking, however, are shown to affect the fluorescence reabsorption.

## Introduction

The emission properties of many chromophores are responsive to the nature of their near and distant environments. Thus, we have the exogenous-agents-induced alterations of chloroplast fluorescence emission spectrum at 77°K. In this article a mutation-induced chloroplast fluorescence spectrum change is reported. In addition, the effect of membrane stacking on fluorescence reabsorption is discussed.

The ac-5 mutation in Chlamydomonas reinhardi (Goodenough and Staehelin, 1971) produces cells that are chlorophyll deficient, having higher rate of $CO_2$ fixation per chlorophyll-wise in comparison to wild type cells, implying smaller photosynthetic unit. The mutant cells can be grown either phototrophically on a minimal medium or mixotrophically in the light on a minimal medium supplemented with acetate; under both growth conditions the cells are equally

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pigment deficient and with comparable photosynthetic capacity. Fine structure examination reveals striking differences among cells of wild type and the two types of ac-5 mutants under different growth conditions. In wild type cells, thylakoids are usually found in stacks of 2-7 thylakoids. Thylakoids of phototrophic ac-5 cells traverse either singly or more often than not in a group of two. In contrast, mixotrophic ac-5 cells are incapable of any chloroplast membrane stacking. Freeze-etch electron microscopy studies suggest that changes within the membrane may result in the unstacking of thylakoid membrane. In this report, it is suggested that chlorophyll may be used as a natural fluorescence probe for detecting the sign of changes on the molecular level of membranes.

## Materials and Methods

Wild type (strain 137C) and mutant (strains ac-5) cells were grown on minimal medium (Sueoka, 1960) or minimal medium supplemented with 3% sodium acetate. The light intensity from daylight fluorescent lamps was 2000 lux, and the temperature was maintained at 26°C. Either cells or chloroplast fragments prepared by the ultrasonic disruption of cells (Levine and Gorman, 1966) (in a medium containing 50 mM Tricine, pH 7.3 and 150mM sucrose) were used for the fluorescence measurements (Li, 1974). The emission spectra had been corrected for variations with wavelength, in the sensitivity of the photomultiplier and the light transmittance of filters, but not for the efficiency of the monochromator. Except mentioned otherwise, all spectra are normalized at 685 nm.

## Results and Discussion

Fig. 1 compares the low temperature emission spectrum of the wild type cells with those of ac-5 cells under two growth conditions. The relative value of system I fluorescence (Goedheer, 1968) F715 (fluorescence peak at 715 nm) is doubled in the mutants. One speculates that the state of stacking may be responsible for the change of emission spectrum. However the increase of F715 is not correlated with the degree of stacking of the thylakoid membranes, for the unstacking mixotrophic ac-5 cells have an F715 whose intensity is intermediate between the wild type and the phototrophic ac-5 mutant, both of them have stacking membranes. Therefore, changes within the membranes (Goodenough and Staehelin, 1971), rather than their stacking states, may be responsible for the spectrum alterations. Less conspicuous changes in the spectrum range between 685 nm and 690 nm are not carefully studied. However, they are not as a consequence of differences in chlorophyll concentrations, because a change of concentration from 3.6 to

14 μgchl/ml produces no changes of relative fluorescence in these wavelength range in wild type cells (fig. 6).

The low temperature emission spectra of chloroplast fragments from phototrophic ac-5 cells and the effects of salts and buffer concentration on them are depicted in figures 2 and 3. The *in vivo* proportion of F687 and F715 can be maintained provided that the chloroplasts suspension is buffered and $CaCl_2$ is added. Without the addition of salts the relative F715 is higher than the *in vivo* value. Although the high value of F715/F687 remains unchanged after the removal of most the Tricine buffer from the suspension (fig. 3), the potential F715 response to the salt addition (fig. 2) is lost. The differential responses of F735 to sodium and calcium ions found in spinach chloroplasts (Li, 1974) are not observed here. As salts causes restacking of chloroplast membranes irrespective of the presence of buffer (Izawa and Good, 1966; Spencer and Unt, 1965; Murakami and Packer, 1971), the buffer-dependent salt-induced change of F715 indicates that the fluorescence change is not a result of changes in membrane stacking states. Those observations reveal also that the proton concentration seems to affect the properties of chloroplast membranes. How fluorescence responses to the change of pH and what is the salt effect at each pH value in isolated chloroplasts of membrane

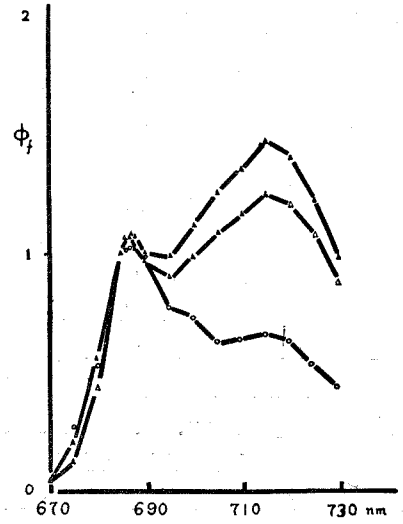


Fig. 1. A comparison of fluorescence emission spectrum of wild type cells with those of ac-5 cells. Cells were suspended in 50 mM tricine, pH 7.3. ○: wild type cells, 3.6 μg chl/ml; △: mixotrophic ac-5 cells, 2.9 μg chl/ml; ▲: phototrophic ac-5 cells, 2.6 μg chl/ml; $\phi$: relative fluorescence yield.

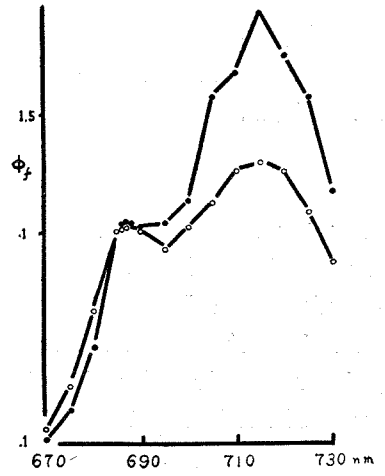


Fig. 2. $CaCl_2$ effects on the low temperature emission spectrum of chloroplast fragments of phototrophic ac-5 cells. Fragments were (0.5 μg chl/ml) suspended in 50 mM tricine pH 7.3. ○: no addition; ●: 5 mM $CaCl_2$; $\phi$: relative fluorescence yield.

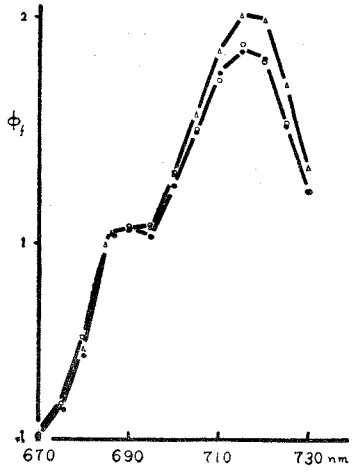


Fig. 3. Effects of salts on the low temperature emission spectrum of chloroplast fragments suspended in low concentration buffer. Fragments (0.5 $\mu$g chl/ml) of phototrophic ac-5 cells were suspended in 500 $\mu$M tricine. ○: 5 mM $CaCl_2$ added; ●: 10 mM NaCl; △: no addition; $\phi$: relative fluorescence yield.

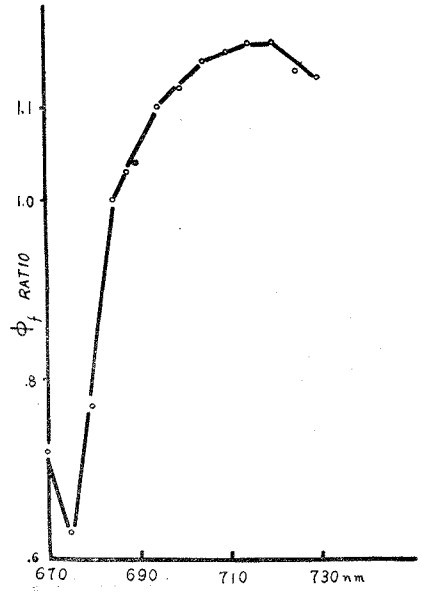


Fig. 4. Fluorescence ratio curve of phototrophic and mixotrophic ac-5 cells (low temperature). Conditions were the same as in figure 1.

mutant and wild typecells are certainly points of interest. Are the fluorescence responses to the salt addition in the isolated chloroplasts of mixotrophic and phototrophic ac-5 cells different because of the different morphological responses (Goodenough and Staehelin, 1971)? In view of the salt-induced change of fluorescence spectrum, one may even ask, where are the emitters? Are they situated in a hydrophilic or a hydrophobic environment? How do the salts influence the hydrophobic interior of membranes?

Although there is no correlation between F715 and the states of membrane stacking, that is not to say that the latter has no effect on the emission spectrum at all. When the ratio of emission spectra of phototrophic and mixotrophic ac-5 mutants is taken and plotted as a function of wavelengthes (fig. 4 and 5), the curves resemble the ratio curves of emission spectra measured from two samples differred only in their chlorophyll concentrations (fig. 6). The partial overlapping of absorption and emission spectra can explain the curves in Fig. 6. Apply Beer-Lambert's law to this situation, one obtain

$$F_o(\lambda)=F_e(\lambda)e^{-\varepsilon_\lambda c}$$

where $F_o(\lambda)$ is the fluorescence intensity observed at wavelength $\lambda$; $F_e(\lambda)$, the intensity emitted at $\lambda$; $\varepsilon_\lambda$, the absorbancy at $\lambda$; $c$, concentration. With

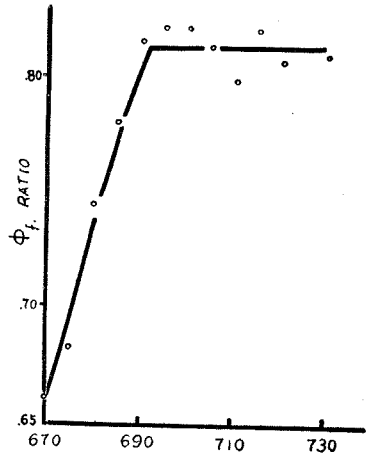


Fig. 5. Fluorescence ratio curve of phototrophic and mixotrophic ac-5 cells (room temperature). Cells (5.5 μg chl/ml) were suspended in 50 mM tricine pH and 7.3 spectra were not normalized.

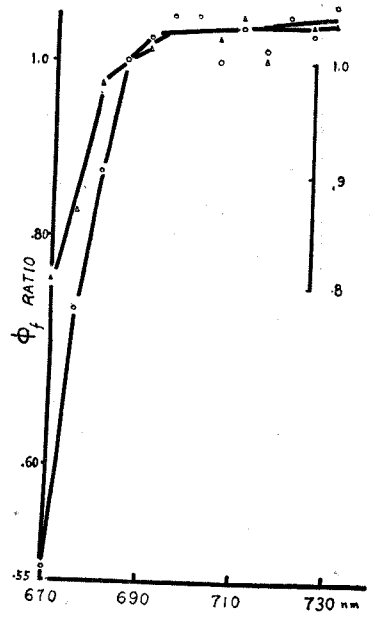


Fig. 6. Fluorescence ratio curves of wild type cell suspensions with different concentrations. Cells suspended in 50 mM tricine, pH 7.3. ○: $\frac{14\ \mu g\ chl/ml}{1.45\ \mu g\ chl/ml}$, (left side scale); △: $\frac{3.6\ \mu g\ chl/ml}{1.45\ \mu g\ chl/ml}$ (right side scale).

two different chlorophyll concentrations the fluorescence ratio is proportional to $e^{-\varepsilon_\lambda d c}$. High reabsorption occurs at short wavelength where the absorption of chlorophyll is high, the reabsorption peters out towards the long wavelengthes and disappears almost completely at about 690 nm ($\varepsilon_\lambda$ approaches zero), this is showing on the curves of Figure 6 as a leveling off of the ratio curve at about 690 nm. If no reabsorption occurs at all wavelength, a straight line would have been observed in Figure 6. In mutant cells, it is interesting to point out that a chlorophyll molecule in an unstacking thylakoid is seeing less chlorophyll molecules around its immediate surroundings than a chlorophyll in stacked thylakoids does. This difference in chlorophyll concentration may extend to the chloroplast as a whole, since with average larger spacing between membranes, it is reasonable to assume that the chloroplast with unstacking membranes has a larger volume than that with stacking membranes; as the mutants under either growth condition have the same amount of chlorophylls (Goodenough and Staehelin, 1971), they are, therefore, less concentrated in the unstacking chloroplast (one cup-shaped chloroplast per

cell). With this in mind, curves shown on Figures 4 and 5 are readily understandable, since in dilute cell or chloroplast suspensions, chlorophyll concentration within chloroplast is a much more important factor in determining the reabsorption than the overall concentration in the cuvette, because the chlorophylls are not evenly dispersed and the distances between cells are large. Figure 5 indicated that there is no fluorescence band shift caused by the reabsorption as reported by French and Young (1952). The small variation of the ratio curve on Figure 4 is not understood.

While how the F715 is changed in ac-5 mutants is not known, the present finding does provide a rationale for a future systematic fluorescence study on other mutants. Furthermore, parallel fine structure study (Goodenough and Staehelin, 1971), biochemical studies and studies on the molecular environment of membrane using exogenous fluorescence probe as well as chlorophyll fluorescence may provide some useful information on the chloroplast membrane structure.

## Literature Cited

BROWN, J. S. and M. R. MICHEL-WELWERTZ. 1968. Chlorophyll fluorescence near 720 mμ in Euglena extracts. Biochim. Biophys. Acta **153**: 288-290.

FRENCH, C. S. and V. K. YOUNG. 1952. J. Gen. Physiol. **35**: 877-

GOEDHEER, J. C. 1964. Fluorescence bands and chlorophyll a forms. Biochim. Biophys. Acta **88**: 304-317.

GOEDHEER, J. C. 1968. On the low-temperature fluroescence spectra of blue-green and red algae. Biochim. Biophys. Acta **153**: 903-906.

GOODENOUGH, U. W. and L. A. STAEHELIN. 1971. Structural differentiation of stacked and unstacked chloroplast membranes. Freeze-etch electron microscopy of wild-type and mutant strains of Chlamydomonas. J. Cell Biol. **48**: 594-619.

LI, Y. S. 1974. The thermal and ion effects on the low temperature emission spectrum of spinach chloroplasts. Bot. Bull. Acad. Sin. **15**:.

LEVINE R. P. and D. S. GORMAN 1966. Photosynthetic electron transport chain of Chlamydomonas reinhardi. III, Light-induced absorbance changes in chloroplast fragments of the wild type and mutant strains. Plant Physiol. **41**: 1293-1300.

MURAKAMI, S. and L. PACKER. 1971. The role of cations in the organization of chloroplast membranes. Arch. Biochim. Biophys. **146**: 337-347.

MURAKAMI, S. and L. PACKER. 1970. Protonation and chloroplast membrane structure. J. Cell Biol. **47**: 332-351.

SPENCER, D. and H. UNT. 1965. Biochemical and structural correlations in isolated spinach chloroplasts under isotonic and hypotonic conditions. Aust. J. Biol. Sci. **18**: 197-210.

SUEOKA, N. 1960. Mitotic replication of deoxyribonucleic acid in Chlamydomonas reinhardi. Proc. Nat. Acad. Sci. U.S.A. **46**: 83-91.

# Chlamydomonas reinhardi 的野生種及一種葉綠體膜變種的低溫螢光放射光譜 兼論螢光的再吸收與葉綠體膜層疊的關係

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放射螢光的分子因其所處的環境不同而改變其放射光譜或强度。葉綠體中葉綠素的螢光也不例外；葉綠體螢光因受人工處理而改變的例子很多。自不同種系植物分離出的葉綠體，其放射的螢光已知各有差異。但研究同一種植物野生種及其變種，特別是葉綠體變種的螢光差異的報告尙付缺如。

本文報導 Chlamydomonas reinhardi 野生種及其一種葉綠體變種 ac-5 的低溫螢光光譜。ac-5 的 F715（尖峯在 715 nm 的螢光）與 F687 的比值高于野生種比值約一倍。這一螢光的改變與變種中葉綠體膜的外觀變異無關，因爲變種的葉綠體膜能因生活環境的改變而呈現不同形態，但其光譜却不隨之作相應的改變。顯然變種膜中微細變異有非外觀所能昭示者。螢光研究的價值正在于其對細微環境改變有較其他一般儀器更爲敏感的辨別能力。將螢光光譜的研究與生化分析或其他工具合併使用，或能有助于人們對葉綠體膜的瞭解。

本文並附帶討論葉綠體膜的外觀變異與其螢光再吸收現象的關係