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## The role of chloride ion in photosynthesis. IV. Studies on the low temperature fluorescence emission spectrum

Maximal rates of noncyclic electron flow in isolated chloroplasts are dependent on the presence of $Cl^-$ at a concentration of about 10 mM[1,2]. Evidence presented in an earlier report[3] suggested that $Cl^-$ functions between the site of oxidation of water and the reaction center of Photosystem II. If this is the case, $Cl^-$ might be expected to influence the low temperature fluorescence emission from Photosystem II, since impairment of electron flow in this same region by $Mn^{2+}$ deficiency has striking effects on fluorescence[4]. The fluorescence emission from $Cl^-$-depleted chloroplasts at liquid nitrogen temperature was therefore studied.

$Cl^-$-depleted chloroplasts were isolated as previously described[1] from spinach ("standard preparation"). EDTA-treated chloroplasts were isolated and prepared as detailed in an earlier report[5]. The fluorimeter was modified so that the sample could be placed in liquid nitrogen[6]. Chloroplasts giving a final concentration of 20 $\mu$g/ml chlorophyll were suspended in the following medium (mM): sucrose (200), $MgSO_4$ (5), tricine–NaOH (30 at pH 8.4) and where indicated NaCl (10). Approx. 1 ml of this mixture was placed in a quartz-glass cuvette (optical depth, 1 mm) and rapidly frozen in liquid nitrogen. The blue exciting light (436 nm) was modulated at 270 Hz and had a mean intensity of 0.5 kerg/cm$^2$·sec. The analyzing monochromator had a band width of 0.7 nm. Correction for the spectral sensitivity of the apparatus was made as previously described[6]. A signal averager was used to average the output signal 128 times at each wavelength.

Fig. 1 shows the fluorescence emission spectra of chloroplasts in the presence and absence of $Cl^-$. The curve obtained with $Cl^-$ present resembles that described

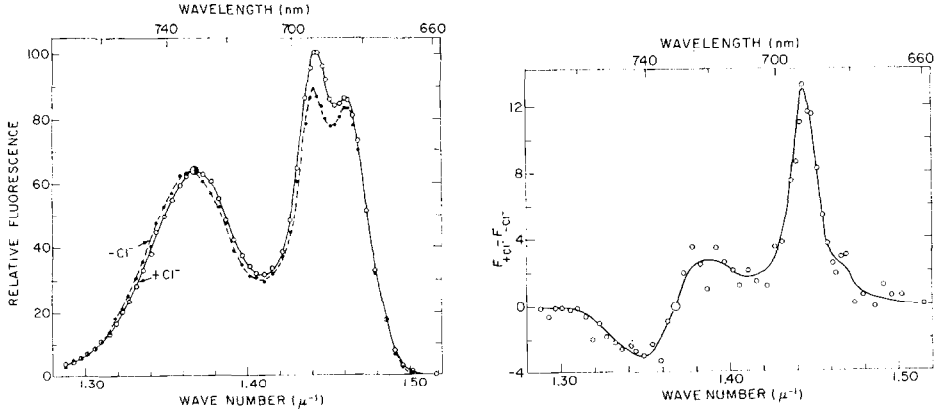


Fig. 1. Low temperature fluorescence emission spectrum for $Cl^-$-depleted chloroplasts. Chloroplasts suspended as in text were frozen with and without NaCl (10 mM) present. Exciting light as in text. Fluorescence at 1.368 $\mu^{-1}$ for $+Cl^-$ and $-Cl^-$ set equal. Fluorescence given in relative quanta per wave number.

Fig. 2. Difference spectrum of low temperature fluorescence emission from $Cl^-$-depleted chloroplasts. Data from Fig. 1; with $F_{+Cl^-}$, $F_{-Cl^-}$ = relative fluorescence per wave number with and without added NaCl.

by GOVINDJEE AND YANG[7]. The peak at 693 nm (1.443 $\mu^{-1}$) is nearly 13% higher than the 684-nm (1.462 $\mu^{-1}$) peak, and is 50% higher than the peak at 731 nm (1.368 $\mu^{-1}$). In the absence of $Cl^-$, the fluorescence yield over the entire spectrum is lowered by 8–12%. The curves in Fig. 1 have been normalized at 731 nm and so do not illustrate this effect. They do show, however, that in the absence of $Cl^-$ the 693-nm peak is lower with respect to the peaks at 684 and 731 nm. The ratio between the peak heights at 684 and 731 nm is scarcely affected by the presence or absence of $Cl^-$.

In Fig. 2, the curves shown in Fig. 1 have been subtracted to give a difference spectrum for fluorescence emission in the presence and absence of $Cl^-$. There is a large peak at 693 nm and possibly a minor shoulder at 684 nm. Also, the difference spectrum clearly shows a $Cl^-$-dependent shift of the 731-nm peak to lower wavelength; giving a difference peak at 722 nm (1.385 $\mu^{-1}$) and a trough at 740 nm (1.350 $\mu^{-1}$).

TABLE I

THE EFFECT OF IONS UPON THE FLUORESCENCE EMISSION OF $Cl^-$-DEPLETED CHLOROPLASTS

Prep. 1: Average of 11 trials from 1 to 6 h after breakage (± error of mean). Chloroplasts (standard preparation) suspended and frozen as in text. *o*-Chlorophenolindo-2,6-dichlorophenol reduction rate: ($+Cl^-$) = 50–150 μequiv/mg·h. $Cl^-$ effect = 3.0–4.0 (ref. 1). Prep. 2: Average of 2 trials except for $+Cl$ (4), using signal averager (± variation) as in Prep. 1. Where indicated, NaF (10 mM), or $Na_2SO_4$ (10 mM). *o*-Chlorophenolindo-2,6-dichlorophenol reduction rate: ($+Cl^-$) = 75, ($-Cl^-$) = 7 μequiv/mg·h. Prep. 3: Average of 4 trials (± error of mean), using EDTA-treated chloroplasts (10 μg chlorophyll/ml) in sucrose (0.1 M), *N*-tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid (0.03 M), $MgSO_4$ (0.005 M), pH 8.1.

| *Preparation* | *Ratio of peak fluorescence* | |
|---|---|---|
| | $F_{693\,nm}/F_{684\,nm}$ | $F_{693\,nm}/F_{731\,nm}$ |
| *Standard* | | |
| 1. $+Cl^-$ | 1.20 ± 0.01 | 1.65 ± 0.05 |
| $-Cl^-$ | 1.11 ± 0.01 | 1.51 ± 0.04 |
| 2. $+Cl^-$ | 1.12 ± 0.02 | 1.58 ± 0.04 |
| $-Cl^-$ | 1.05 ± 0.01 | 1.42 ± 0.02 |
| $+F^-$ | 1.03 ± 0.01 | 1.59 ± 0.01 |
| $+SO_4^{2-}$ | 1.04 ± 0.01 | 1.41 ± 0.02 |
| *EDTA-washed* | | |
| 3. $+Cl^-$ | 1.23 ± 0.01 | 1.23 ± 0.02 |
| $-Cl^-$ | 1.10 ± 0.01 | 1.27 ± 0.01 |

The ratios between the fluorescence emissions at 684, 693 and 731 nm for two different standard chloroplast preparations are given in Table I. EDTA-washed chloroplasts were used in the third experiment. The $F_{693\,nm}/F_{684\,nm}$ ratio was increased by addition of $Cl^-$ in all cases. However, $F^-$ and $SO_4^{2-}$, which are known to be unable to replace $Cl^-$ in electron transport studies[1], are unable to increase this ratio.

By contrast, the $F_{693\,nm}/F_{731\,nm}$ ratio is increased 9–13% in the presence of $Cl^-$, with the standard chloroplast preparation; but is decreased slightly with the EDTA-treated preparation. Furthermore, there is a significant increase in this ratio upon addition of $F^-$, but no effect upon the addition of $SO_4^{2-}$. The ratio of $F_{684\,nm}/F_{731\,nm}$ (data not shown) is likewise unresponsive in a reproducible and

specific fashion to the effects of $Cl^-$ addition. These observations *plus* the higher standard deviation of ratios involving $F_{731\ nm}$, lead to the conclusion that $F_{731\ nm}$ is too sensitive to unknown factors to be used as a reliable reference in studies with $Cl^-$-deficient chloroplasts.

The low temperature fluorescence at 693 nm is thought to indicate the state of the Photosystem II trap, whereas the 684-nm emission is assigned to bulk chlorophyll[7,8]. The fluorescence at 731 nm seems to be related to the Photosystem I trap[8]. The presence of $Cl^-$ increases the yield of all fluorescent forms of chlorophyll *a*; perhaps by altering the structural state of the thylakoid, or the aggregation of chlorophyll during freezing[9]. The selective enhancement of $F_{693\ nm}$ in the presence of $Cl^-$ may indicate a shift in the redox state of the Photosystem II trap prior to freezing. This interpretation would be in accord with our proposed location for the site of $Cl^-$ function between the water-splitting reaction and the photoact of System II (ref. 5).

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