# Reabsorption of Chlorophyll Fluorescence and Its Effects on the Spectral Distribution and the Picosecond Decay of Higher Plant Leaves

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Effects of reabsorption of chlorophyll fluorescence were investigated in measurements on chloroplasts and higher plant leaves. The excitation was performed at 650 nm in a typical excitation/detection arrangement; with increasing chlorophyll concentrations, the measured spectral distribution of the chlorophyll fluorescence was significantly distorted, but no effects on the picosecond decay were observed, when this was detected at 685 nm.

## Introduction

Reabsorption of fluorescence occurs when the absorption spectrum and the fluorescence spectrum overlap. Fluorescence photons, emitted after the initial excitation, are possibly reabsorbed and re-emitted as fluorescence again, distorting the measured spectral distribution of fluorescence and increasing the measured fluorescence decay times. Many factors, for example the excitation/detection arrangement and the concentration of molecules, can amplify these effects (Birks, 1970; Sakai *et al.*, 1989; Ahmed *et al.*, 1994).

In photosynthesis research it is well known that the measured spectral distribution of chlorophyll fluorescence depends on the pigment concentration. The ratio of the chlorophyll fluorescence intensities in the maxima at about 685 nm and 740 nm varies between 4 to 5 in dilute chloroplast suspensions and 0.5 to 1 in leaves, due to the effects of reabsorption (Hák *et al.*, 1990; Lichtenthaler *et al.*, 1990). In view of these results it is interesting to note that reabsorption effects were not discussed in chlorophyll fluorescence decay measurements, that appeared in the literature until now. Because theoretical predictions of the reabsorption effects are complicated by the irregular distribution of chlorophyll molecules, by scattering of excitation light and fluorescence photons, and other factors, it is more reliable to obtain experimental evidence by combined measurements of the spectral distribution of chlorophyll fluorescence and the corresponding chlorophyll fluorescence decay times.

## Materials and Methods

Measurements were carried out using leaves of spinach (*Spinacea oleracea* Atlanta). Chloroplasts were prepared according to Walker *et al.* (1987). Leaves and chloroplasts were treated with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) to block all photosystems II (PS II): leaves were put into a $10^{-3}$ M aqueous solution for about 15 min and the chloroplast suspensions were supplied with DCMU to give a $10^{-4}$ M concentration. This treatment maximized the chlorophyll fluorescence decay times (Terjung *et al.*, 1997) and enabled a better resolution of the reabsorption effects than in the open state of PS II with the shorter chlorophyll fluorescence decay times. Chlorophyll concentrations of the chloroplast suspensions were determined after extraction in acetone, using the procedure and equation of Bruinsma (1961).

Excitation pulses (650 nm, pulse width of about 15 ps FWHM) were generated by a synchronously pumped and capity-dumped dye laser-system (Spectra Physics 3000) with a repetition rate of 800 kHz and an intensity of about 2 W/m$^2$. Leaves and chloroplasts were excited under the angle of incidence of 50°, fluorescence was observed under the angle of detection of 40°, one of the excitation/detection arrangements typically used in measurements on leaves. The fluorescence detection system consisted of a double monochromator (Jobin Yvon H25) and a multichannel plate-photomultiplier (Hamamatsu R1564u-07). The time response of the setup was about 70 ps. The spectral sensitiv-

*Abbreviations:* DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; FWHM, full width at half maximum; PS II, photosystem II.

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ity of the detection system was corrected using the fluorescence standard Oxazine 1 (3 $\mu$M) in ethanol. After the measurement of the spectral distribution, the chlorophyll fluorescence decay was detected at the wavelength of 685 nm by the method of inverse time-correlated single photon counting (O'Connor and Phillips, 1984). The chlorophyll fluorescence decay was not detected at 740 nm due to the low fluorescence intensities at this wavelength in measurements on chloroplasts. Decay curves were analysed by iterative deconvolution techniques (Marquardt algorithm). The quality of the fits was checked by the reduced chi-square criterium $\chi^2_{red}$ and the random distribution of the weighted residuals. All decay curves were fitted with three exponential functions with $\chi^2_{red}$ between 1 and 1.1. The obtained amplitudes $\alpha_i$ and lifetimes $\tau_i$ were used to calculate the average chlorophyll fluorescence decay time $\tau_m$:

$$\tau_m = \frac{\Sigma_i \alpha_i \tau_i^2}{\Sigma_i \alpha_i \tau_i} \quad (1)$$

## Results and Discussion

The chlorophyll concentrations of the chloroplast suspensions and the average chlorophyll fluorescence decay times of the chloroplast suspensions and the leaf are given in Table I. The measured and corrected spectral distributions of the chlorophyll fluorescence of the chloroplasts and the leaf are shown in Fig. 1. The effects of reabsorption are clearly observed in the spectral distributions and in the shift of the fluorescence maximum around 685 nm, but not in the average chlorophyll fluorescence decay times. Since the error of the average chlorophyll fluorescence decay time is about 5%, no significant distortions due to reabsorption effects were observed. This was also observed for each of the three fluorescence decay components (data not shown). These measurements were repeated three times and gave the same results.

Table I. The chlorophyll concentrations of the chloroplast suspensions (Chl) and the average chlorophyll fluorescence decay times, detected at 685 nm ($\tau_m$), in comparison to the data of a leaf.

| Chloroplast suspension No. | Chl [$\mu$g Chl/ml] | $\tau_m$ [ns] |
|---|---|---|
| 1 | 3 | 2.27 |
| 2 | 10 | 2.27 |
| 3 | 59 | 2.20 |
| 4 | 319 | 2.32 |
| Leaf | – | 2.40 |

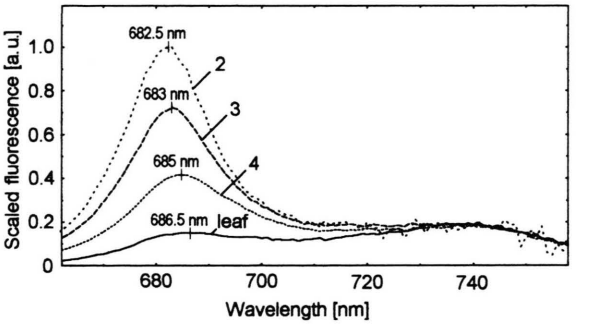


Fig. 1. The spectral distribution of the chlorophyll fluorescence to the data of Table I; the numbers correspond to the chloroplast suspension numbers of Table I. All spectra were scaled to the same fluorescence intensity around 740 nm. For chloroplast suspension No. 1, the fluorescence intensity around 740 nm was too low for the measurement of the complete spectral distribution.

These measurements point out, that the chlorophyll fluorescence decay, excited at 650 nm under the angle of incidence of 50° and detected at 685 nm under the angle of detection of 40° is not significantly distorted, corrections for reabsorption effects are not neccessary. This is a remarkable advantage compared with measurements on the spectral distribution of chlorophyll fluorescence.

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Ahmed S. A., Zang Z. W., Yoo K. M., Ali M. A. and Alfano R. R. (1994), Effect of multiple light scattering and self-absorption on the fluorescence and excitation spectra of dyes in random media. Appl. Opt. **33**, 2747–2750.

Birks J. B. (1970), Photophysics of Aromatic Molecules. Wiley-Interscience, London, New York, Sydney, Toronto.

Bruinsma J. (1961), A comment on the spectroscopic determination of chlorophyll. Biochim. Biophys. Acta **52**, 576–578.

Hák R., Lichtenthaler H. K. and Rinderle U. (1990), Decrease of the chlorophyll fluorescence ratio F690/F730 during greening and development of leaves. Radiat. Environ. Biophys. **29**, 329–336.

Lichtenthaler H. K., Hák R. and Rinderle U. (1990), The chlorophyll fluorescence ratio F690/F730 in leaves of different chlorophyll content. Photosynth. Res. **25**, 295–298.

O'Connor D. V. and Phillips D. (1984), Time-Correlated Single Photon Counting. Academic Press, London.

Sakai Y., Kawahigashi M., Minami T., Inoue T. and Hirayama S. (1989), Deconvolution of non-exponential emission decays arising from reabsorption of emitted light. J. Lumines. **42**, 317–324.

Terjung F., Berg D., Maier K. and D. Otteken (1997), Investigations on the electron transfer processes in photosystem II: New insights by chlorophyll fluorescence decay measurements with additional saturating light pulses. Photosynth. Res. **53**, 29–34.

Walker D. A., Cerovic Z. G. and Robinson S. P. (1987), Isolation of intact chloroplasts: General principles and criteria of integrity. Methods Enzymol. **148**, 145–157.