Regulation of Light Energy Distribution between Photosynthetic Pigment Systems; a Possible Role of Leaf Anatomy

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Photosynthetic Pigments, Energy Distribution, Leaf Anatomy

The complex anatomical structure of an intact leaf results in a distribution of photosynthetically active energy between photosynthetic pigments which is different from that observed in isolated chloroplasts. The variance is due mainly to scattering at the gas-liquid interface between cells and intercellular space which tends to increase light absorption by the long wavelength absorbing pigments through secondary fluorescence. Evidence is given in support of an active use of this feature by the higher plant to regulate energy flow at the photophysical level of light absorption.

Introduction

The complex system of leaf structure has been viewed so far only as a means to achieve an optimal balance between transpiration, respiration and distribution of nutrients and products in relation to photosynthetic capacity. Little emphasis has been placed on the logical consequence, that this balance is a dynamic one being in constant need for adjustment.

Since photosynthesis is a key factor for the maintenance of overall metabolic stability it can be assumed, that the chloroplast is the main lever for the pertinent regulatory mechanisms. A variety of such systems are known, ranging from those acting at the pigment level (*e.g.* redistribution through spillover and dissipation by fluorescence) to those influencing the Blackmann reaction through modulation of gas exchange.

Investigations of regulatory features at the pigment level have so far used isolated chloroplasts as experimental system and resulted in a coherent model of interactions between several pigment systems [1]. Key parameter in many of these experiments was the measurement of the fluorescence energy emitted at 77 °K from the various pigment species making up the different pigment complexes.

The interpretation of these results to the *in vivo* situation tacitly assumes that the organization of chloroplasts within the living leaf and the structural features of this plant-organ itself are of no or only minor consequence for the proposed functioning of the pigment-level regulation. Although experiments concerning the conformational change of plastids *in vivo* [2] appeared to bear out this conclusion, it remains still a disputable notion.

It seemed, therefore, warranted to investigate the possible participation of leaf structure on the energy distribution of absorbed light. The results are reported in the following sections.

Materials and Methods

Leaves of 6 week old tobacco plants, grown in the greenhouse, were used for the fluorescence measurements without further processing. Their chlorophyll content, given per unit area, was determined according to Arnon [3].

Whole chloroplasts were prepared using the method of Nakatani and Barber [4]. The isolation medium contained 0.33 M sucrose, 0.2 mM $MgCl_2$ and 20 mM MES pH 6.5, the final suspension medium 0.33 M sucrose – 0.5 mM TRICINE pH 7.5. Both the tobacco leaves and the chloroplast suspensions were dark adapted for 15 min before use.

For the measurements of leaf fluorescence in relation to time of day, dark adaption was omitted. The material for comparative measurements of various plant species was obtained in the Göttingen botanical garden.

The fluorescence spectra were determined at room temperature using the instrumental setup described earlier [5]. The leaf was illuminated with

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the aid of one arm of a double arm fiber optic, the fluorescence was taken at 360° from the excitation using the other. Chloroplast suspensions were put into a petri-dish. The illumination and fluorescence measurements were made through its bottom using the same setup.

Excitation was between 475 – 500 nm with an intensity of 1 mW × cm^{-2}. The fluorescence was scanned between 660–760 nm. The resulting spectra were corrected against filter tailing, monochromator-aberrations and photomultiplier sensitivity using appropriate blanc-samples and calibration curves. The standard deviation, determined in series experiments using 10 pieces of individual tobacco leaves, was 7%.

Results

A comparison of the fluorescence emission spectrum of a whole leaf section and that of a chloroplast suspension, adjusted to the same chlorophyll content per illuminated area and the same thickness, shows a considerable amount of emission in the long wavelength region for the former while its fluorescence band around 690 nm is appreciably lower (Fig. 1). The area under the curves, a measure of the total amount of energy emitted, is approximately equal. Obviously, a shift of emission from the pigment systems takes place due to structural features of the leaf itself.

Starting with the hypothesis, that this observation rests basically on the phenomenon of secondary fluorescence (reabsorption of energy emitted at 690 nm by the chlorophylls fluorescing at 742 nm) and is not caused by intrathylakoidal redistribution of energy, attempts were made to model this behaviour. The data of Fig. 2 exclude, that a simple packing of plastids, *i.e.* an increase of secondary fluorescence by enlarging the probability for reabsorption of emitted energy, accounts for the size of the phenomenon. Taking the ratio F_{742}/F_{690} as a measure, dense packing can effect only half of the value of 0.8 observed *in vivo*.

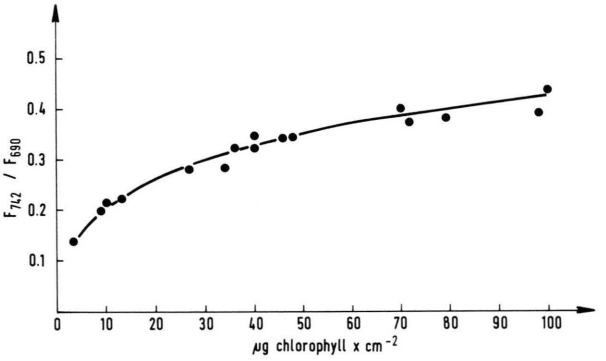

Fig. 2. Influence of chloroplast density per illuminated area on the ratio of fluorescence emission F_{742}/F_{690} at room temperature. Suspension layer thickness 1.6 mm; the corresponding ratio of the intact leaf was 0.8 at 51 µg chlorophyll × cm^{-2}.

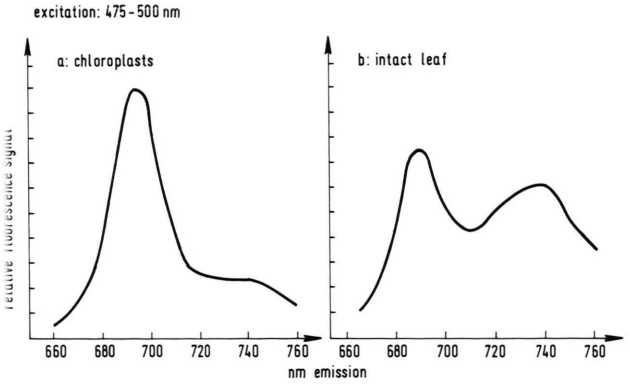

Fig. 1. Room temperature fluorescence emission spectrum of chloroplasts as compared to that of an intact leaf. The chloroplast suspension was adjusted in chlorophyll content and thickness of the suspension layer to the values of the leaf.

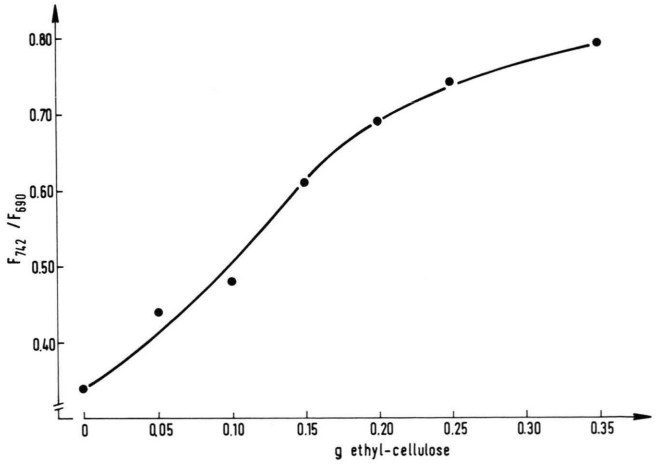

Fig. 3. Increase of fluorescence ratio F_{742}/F_{690} of isolated chloroplasts through addition of ethylcellulose. Chlorophyll concentration was 51 µg/cm^2, suspension layer thickness 1.6 mm. The concentration of ethyl-cellulose is given per 3 ml total suspension volume.

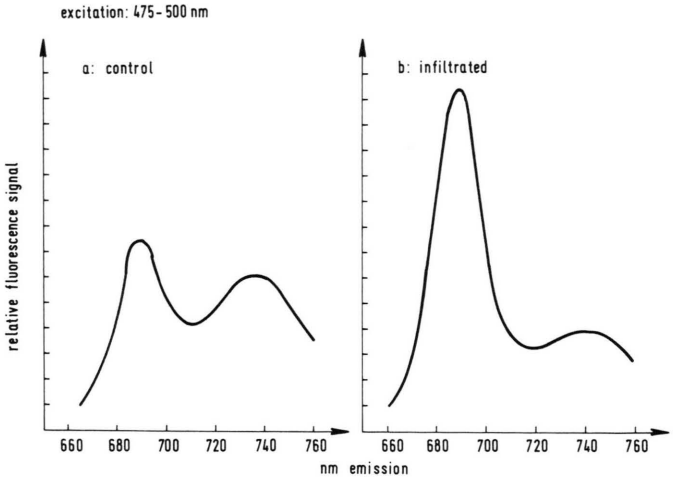

Fig. 4. Comparison of room temperature fluorescence emission spectra of an intact tobacco leaf before (control) and after infiltration with water.

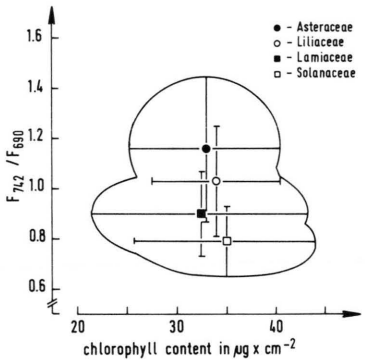

Fig. 5. Graphic summary of fluorescence emission ratios F_{742}/F_{690} at room temperature of intact leaves belonging to species of the various families indicated. Between 6 and 15 species of every plant family, all leaves with similar anatomical features par family, were used. The standard deviation is given by the respective bars.

A major share of the observed increase in secondary fluorescence must thus be attributed to scattering on non chloroplast material of the leaf. The experiments depicted in Fig. 3, modeling the *in vivo* situation by using increasing amounts of fibrous ethylcellulose, indicate clearly, that the increase in emission ratio is dominated by this feature. Scattering in the leaf appears to be caused largely at the interphase between gas and liquid, namely the border between cells and intercellular space. A simple proof for this reasoning is available. Abolishing the phase boundary by infiltrating the leaf with water under gentle evacuation should and does result in a change of the fluorescence spectrum towards that observed with isolated plastids (Fig. 4).

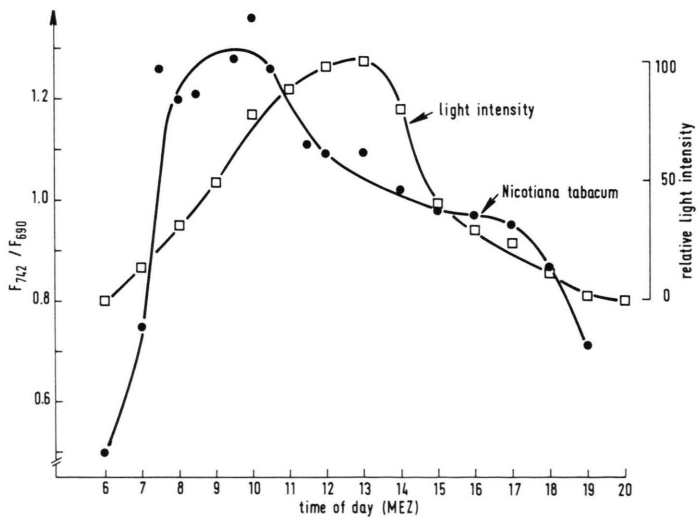

Fig. 6. Variation of the room temperature fluorescence ratio F_{742}/F_{690} of a tobacco leaf in relation to sun-light intensity and time of day. Tobacco plant of Göttingen botanical garden; measurements without dark adaptation.

Table. Fluorescence emission ratios F_{742}/F_{690} of various monocotyledons showing the C_4-pathway of carbon assimilation.

Species	Ratio
Saccharum officinale	0.67
Sorghum bicolor	0.58
Sorghum cernum	0.55
Zea mays	0.35

The experimental results confirm thus the close relation between the emission ratio and the structure of the leaf.

To strengthen this conclusion, leaves of 41 plant species of the Göttingen botanical garden, belonging to 5 different families and taken at random, where checked accordingly. The presence of uniform anatomical features within a family was assessed before-hand with microscopic techniques. Fig. 5 shows the summary of these measurements for 4 families of dicotyledons.

Within the variations depicted, a correlation between the closely related species of a family and the emission ratio seems to be manifest. The data of C-4 plants with their bundle sheath structure range much lower (Table) and are in line with this reasoning.

The data are the starting point for the hypothesis that a change in leaf structure, presumably a shift in the extend of interface scattering due to stomatal regulation and leaf-water content, may influence photosynthetic metabolism. The results of Fig. 6 support such a feature. The emission ratio taken throughout the day varies in much the same manner as the photosynthetic activity in the experiments of Lange [6, 7].

Discussion

The existence of a sizeable long wavelength emission at room temperature in whole leaves as compared to its very low intensity in isolated chloroplasts reflects primarily the responsibility of secondary fluorescence for the former. The experimental findings are congruent with data by Virgin [8], who used chloroplast soaked glass-wool as experimental model. They underline the fact, that the complexity of leaf-structure, esp. the physical parameters scattering, diffraction, refraction due to the existence of cell organelles, cell walls and gas-filled intercellular spaces, has to be included in assessing the energy distribution within the photosynthetic pigment system of higher plants. The distortion of spectral intensities compared to the isolated plastids is in the order of magnitude of those observed by Murata at 77 °K [9]. It should however be remembered, that in the latter case an artificial system (isolated chloroplasts, addition of ions, low temperature) has been used and that the interpretation of its causes and its relevance to the *in vivo* situation is radically different.

The notion, that leaf anatomy influences the energy distribution within the photosynthetic apparatus, is by itself neither new (*e.g.* [10]) nor particularly interesting. It gains, however, in importance if a link can be established between energy distribution and photosynthesis which is actively altered according to metabolic demand through subtle changes in leaf anatomy. Circumstantial evidence has been forwarded in this paper, which is in concordance with data reported by Björkman [11] and Lichtenthaler [12] on sun- and shade leaves. However, more experimental work is clearly warranted to establish this point.

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