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Zero-phonon transitions of chlorophyll *a* in mature plant leaves revealed by spectral hole-burning method at 5 K

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After monochromatic exposition in the 680–700 nm region persistent holes are formed in the fluorescence excitation spectra of green maize leaves. These narrow (up to 1 cm^{-1}) holes correspond to zero-phonon lines whose intensity may reach 45% of the total fluorescence. Thus the fluorescence bands F_{685} and F_{695}, originating mainly from Photosystem II (Rijgersberg, C.P., Amesz, J., Thielen, A.P.G.M. and Swager, J.A. (1979) Biochim. Biophys. Acta 545, 473–482), are essentially of purely electronic nature. Unlike chlorophyll *a* in glassy matrices, the pigment in greening leaves cannot be completely phototransformed under high-dose Kr^+ laser irradiation at 676.4 nm.

The spectra hole-burning technique has been applied to the reaction centers (RC) of both plants [1–5] and bacteria [6–8]. The same method has revealed that strongly associated pigment pools, algal phycobilines [9,10] and photoactive protochlorphyllide in etiolated leaves [11] retain zero-phonon lines of a considerable intensity. We have shown recently that rather deep (more than 20%) holes can be burnt in the fluorescence and excitation spectra of the long-wavelength (700–745 nm) chlorophyll (Chl) *a* forms in greening plants [12]. In the present paper, selective phototransformations of the (antenna) pigments of mature leaves in the 680–700 nm region are reported.

The fluorescence spectrum of a mature leaf in case of the Soret band excitation is displayed in Fig. 1. The relative intensity of the short-wavelength band system is lower by a factor of approx. 5 in comparison with that for an optically thin suspension of chloroplasts not affected by reabsorption effects [13].

Spectral holes formed after an exposure by a light dose of about 20 $J \cdot cm^{-2}$ and detected in the excitation spectra are shown in Fig. 2. On using the registration wavelength ($\lambda_f = 692$ nm) near the $\lambda_{burn} = 687$ nm, the holedepth reaches 45% of the initial intensity. Under a moderate nonsaturating exposition (1 $mW \cdot cm^{-2}$, 10 min) the hole widths are laser-line-limited and do not exceed 1 cm^{-1}. In general, the holes in the absorption spectra of leaves, cells, chlorophyll-pigment complexes, etc. [1–4,12] are shallower than those measured in fluorescence excitation spectra. This could be explained bearing in mind that in strongly interacting heterogeneous pigment systems of a photosynthetic membrane absorption gets contribution from the lowest $S_1 \leftarrow S_0$ transition which is affected by hole burning [12] and its phonon wing as

Abbreviations: PS, photosystem; Chl, chlorophyll; RC, reaction center; DWF, Debye-Waller factor; CT, charge transfer.

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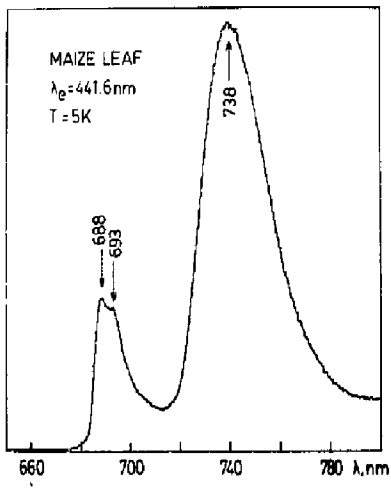

Fig. 1. Fluorescence spectrum of mature maize leaf at 441.6 nm Cd laser excitation at 5 K. The spectrum is corrected for the sensitivity of recording system, which consisted of a DFS-24 double monochromator and a RCA C31034A-02 photomultiplier. The plant was germinated and kept in the dark (7 days) and then exposed 10 days to daylight at 22–25 °C. The fluorescence-band maxima are marked with arrows.

well as from higher $S_n \leftarrow S_0$ (excitonic) energy levels. On the other hand, only $S_1 \rightarrow S_0$ transition with its phonon wing appears in fluorescence at low temperatures. Under definite assumptions (the width of the phonon wing is less than that of the site-energy distribution function, the electron-phonon interaction strength is the same for $S_1 \rightarrow S_0$ and $S_1 \leftarrow S_0$ transitions, etc.) the relative depth of a saturated hole in the excitation spectrum is equal to the Debye-Waller factor (DWF) [14]. It is quite probable that for certain antenna complexes the DWF is higher than for monomeric Chl *a* imbedded in glassy hosts.

There seems to be no definite evidence that RCs can contribute to the fluorescence of chloroplasts. Recently, the hypothesis about F_{695} band being emitted from the acceptor pheophytin molecule of PS II RC was essentially refuted on the basis of thorough spectral studies of chlorophyll proteins of PS II [15]. It means that the zero-phonon lines detected in fluorescence excitation spectra correspond solely to the electronic transitions occurring in the antenna pigment pool.

It is quite a complicated task to differentiate between the holes in absorption originating from RC constituents and antenna pigments. The holes burnt at 700 nm in the absorption spectra of *Chlamydomonas* mutant cells with Chl *a*/RC I ratio of approx. 25 are narrower than 0.11 cm^{-1} [1–3]. Similarly, difference absorption spectra of PS I pigment protein enriched with RC before and after a monochromatic laser excitation in 700 nm region showed a broad-band depletion accompanied by a narrow dip of about 2% depth and of 0.12 cm^{-1} width at 1.6 K [4]. Later the same authors have established that the sharp dip was even narrower (approx. 0.05 cm^{-1}), which provides the relaxation time approx. 200 ps [16]. In both cases, the corresponding lifetimes may be too long to be accounted for as the primary charge-transfer rates in RC [16]. Alternatively, if the narrow features are due to the antenna pigment resonant with RC, the pertinent times would correspond to the energy transfer rather than to the charge separation rate. Obviously, a parallel consideration of absorption, fluorescence and excitation data is necessary for the proper assignment of holes.

Another conclusion, which can be drawn solely from the fact of the existence of strong zero-phonon lines, concerns the impossibility of charge-transfer (CT) character of electronic transitions as a cause of spectral red-shifts of native Chl *a* [17]. The CT inevitably leads to a large Stokes shift (displacement of the minima of potential surfaces) of S_1 and S_0 states, to the diminishing of DWF and to the disappearance of zero-phonon lines (holes) [18]. On the contrary, very broad 'holes' in the case of bacterial [6–8], PS I [4] and PS II [5] RCs as well as recently observed spectral Stark shifts [19] lend support to the CT nature of the electronic transition in primary electron-donor particles.

Rather low light doses (0.1 $mW \cdot cm^{-2}$ during 5 min) can produce remarkable holes in certain spectral regions of the green leaf, e.g., a dip of 10% depth was obtained at $\lambda_{burn} = 688$ nm (λ_f was 693 nm). Such an efficient burning is comparable with the one for the diluted solutions of Chl *a* in glassy matrices where the quantum yield was estimated to be about 10^{-4} [20]. In the monomeric solution of Chl *a* in an organic amorphous host of ether-*n*-butanol, a light dose of 360 $J \cdot cm^{-2}$ (0.2 $W \cdot cm^{-2}$ during 30 min) converts practically

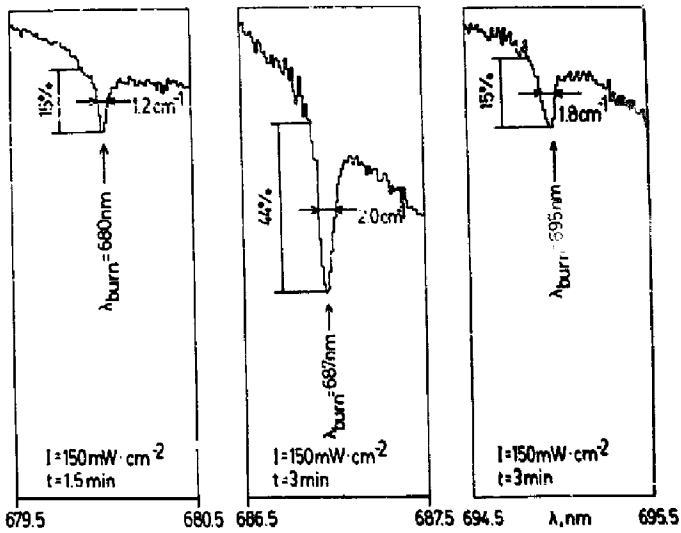

Fig. 2. The excitation spectra of the same leaf as in Fig. 1 with the holes burned at 680, 687 and 695 nm with energy density I during time t; detection was set at 685, 692 and 700 nm, respectively; slit-width was 0.2 nm. A Coherent CR-490 dye laser (line width, 0.5 cm^{-1}) with DCM dye pumped by CR-3 Ar^+ laser was used.

all the centers absorbing through their O–O lines and phonon wings to the centers with the $S_1 \leftarrow S_0$ energy higher than that of λ_{burn} [20] (this was first reported in Ref. 21). The burning in this case is essentially a physical rather than a chemical phenomenon consisting in the conformational motions of the pigment macrocycle, its flexible substituents and host molecules. On the other hand, on an intensive irradiation (8 $W \cdot cm^{-2}$ during 20 min) of a greening leaf, placed in liquid He to avoid its heating with the 676.4 nm Kr^+ laser line, an overall increase of fluorescence was observed (Fig. 3). Therefore, it is possible that after such a harsh treatment some photodesaggregation of the pigment occurs. Despite the fact that additional mechanisms are conceivable as hole-burning pathways in chloroplasts (the weakening of pigment–pigment interactions, the photo-oxidation of antenna pigments with acceptor components of electron-transfer chain, etc.), a considerable amount of native Chl *a* is not phototransformable.

The data presented above and those published recently [12,22] show that narrow (less than 1 cm^{-1}) zero-phonon S_1–S_0 transitions of antenna Chl *a* exist in the spectral interval of 660–745 nm in greening and mature plants. Further, hole-burn-

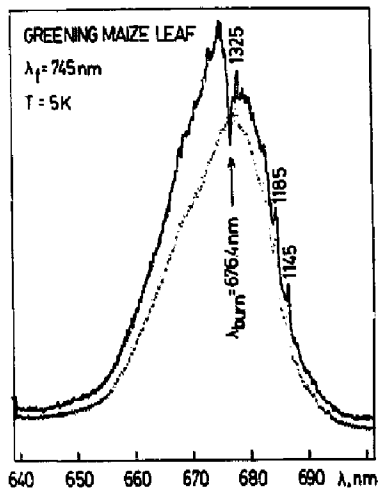

Fig. 3. Fluorescence excitation spectra of a greening maize leaf at 5 K for 745 nm registration (slit-widths 0.3 nm) before (dots) and after (solid line) intensive burning with 676.4 nm Kr^+ laser line (8 $W \cdot cm^{-2}$, 20 min). The plant was germinated and kept in the dark during 10 days, then exposed for 10 h to the daylight. The spectra display purely electronic quasi-lines arising as a result of narrow-band detection in $S_1 \rightarrow S_0$ vibronic region. Ground-state frequencies of Chl *a* are indicated in cm^{-1}.

ing studies should be useful in examining the low-temperature singlet-energy transfer between different Chl *a* forms. Knowing the saturated holedepth in the absorption spectrum and the Debye-Waller factor, the contribution of $S_1 \leftarrow S_0$ could be explicitly distinguished from the $S_n \leftarrow S_0$ absorption of higher excitonic components. From the temperature dependence of the holewidth the values of S_n–S_1 splitting can be estimated.

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