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A spectral hole-burning study of long-wavelength chlorophyll *a* forms in greening leaves at 5 K

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Persistent holes have been burnt in the spectra of post-etiolated maize leaves in the spectral interval 700–745 nm at 5 K. Narrow (< 1 cm^{-1}) holes detectable both in fluorescence and excitation spectra correspond to the zero-phonon purely electronic S_1–S_0 transition of chlorophyll *a* rather than to the $S_1 \leftarrow S_0$ vibronic or $S_n \leftarrow S_0$ higher singlet transitions. The spectral composition of the inhomogeneously broadened absorption and fluorescence contours of long-wavelength chlorophyll *a* forms in plants is discussed in terms of the contributions of phonon-wings, vibronic satellites, purely electronic S_1–S_0 transitions and its higher excitonic components.

Long-wavelength chlorophyll *a* form; Low-temperature spectroscopy; Spectral hole burning; Zero-phonon transition; *(Zea mays)*

1. INTRODUCTION

Optical spectra of chlorophylls (Chls) as integral constituents of photosynthetically active organisms and those of extracted pigments in diluted solutions display remarkable differences. Particularly, in higher plants the strongest fluorescence band peaking at ~740 nm at low temperatures is red-shifted by ~1400 cm^{-1} as compared with the monomeric Chl *a* fluorescence maximum in solutions. Such red-shifts are ascribed to pigment-pigment [1–4] or pigment-protein [5] interactions, which have been substantiated by theoretical [3,4] and experimental [1,2,4] studies of various model systems. In spite of numerous spectroscopic investigations of intact samples and chlorophyll-protein particles [6–10], many important questions are still of current interest, e.g. those concerning the positions of electronic and vibronic levels of pigments in vivo, the heterogeneity of spectral bands, the strength of vibronic and electron-phonon coupling, etc.

Low-temperature laser spectroscopy has provided extensive information about the electronic and vibrational energies of chlorophylls in frozen organic solvent hosts [11]. It has been shown that a number of more complicated systems, such as native phycoerythrin [12], reaction centers of *Chlamydomonas* [13], photoactive protochlorophyllide-holochrome [14] and photosystem (PS) I preparations from higher plants [15], retain zero-phonon lines appearing as narrow holes in the spectra after selective phototransformation of absorbing species under monochromatic illumination.

In this study, we have succeeded in burning narrow and deep holes in the spectra of long-wavelength Chl *a* (absorbing at $\lambda > 700$ nm) on maize leaves in late stages of greening. It has been demonstrated that at 5 K resonant zero-phonon transitions occur both in fluorescence and absorption even at 745 nm, while the coupling of the $S_1 \longrightarrow S_0$ electronic transition with matrix phonons

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and intermolecular vibrations is not essentially stronger than in monomeric Chl *a*.

2. EXPERIMENTAL

Maize seeds were grown in the dark at 25°C for 10 days. After being exposed for 2 days at daylight (1 $mW \cdot cm^{-2}$) they turned remarkably green. A piece of a leaf was placed between quartz plates and cooled in a liquid He cryostat for a few minutes at 5 K. Excitation was performed with an oxazine 1 dye laser pumped by a Coherent CR-2000 Kr^+ laser. The dye laser was tuned in the 700–780 nm region by a mechanical rotation of a Lyo filter consisting of 3 quartz plates. Placing 2 plain-parallel quartz etalons into the cavity enabled one to reduce the linewidth from δ = 0.6 cm^{-1} to less than 0.06 cm^{-1}, rendering the laser continuously non-tunable. The dye laser output was kept constant by a Coherent model 307A noise reduction system. The registration system consisted of a double grating DFS-24 monochromator, a RCA C31034A-02 Peltier-cooled red-sensitive photomultiplier functioning in a single photon-counting mode and a Nokia LP-4900B multichannel analyzer. Fluorescence spectra were corrected for the spectral sensitivity of the spectrometer system.

3. RESULTS AND DISCUSSION

The fluorescence spectrum of a greening maize leaf at 5 K (fig.1, curve 1) is very similar to the one of intact chloroplasts [8], showing that both photosystems and their antennas are fully developed. The 736 nm fluorescence band arises mainly from the antenna connected with PS I although at $700 < \lambda < 735$ nm the fluorescence intensity of PS II [16] and light-harvesting Chl *a/b* protein [17] is also significant.

After 5 min exposure to the 20 $mW \cdot cm^{-2}$ intensity a slight decrease (1–2%) occurred in the absorption of the leaf (the burning wavelength λ_{burn} was set at 725 nm). However, in the excitation spectrum, when recording was set only 5 nm from λ_{burn}, the hole-depth reached 22% of the initial intensity. In this way holes can be registered (fig.2a) within the spectral interval spreading from 700 to 745 nm (fig.1, curve 2). Rather deep holes (~10%) were observed also in fluorescence emission spectra when the excitation wavelength (λ_e) was shifted upwards from λ_{burn} by 5 nm (fig.2b). A hole narrowing by a factor of ~2 (fig.2a, curve 1) was seen on burning with a single-frequency laser and recording the excitation spectrum with a broad-band laser (δ = 0.6 cm^{-1}). Thus, the real holewidth must be less than 0.6 cm^{-1}. The appearance of a hole in both absorption and fluorescence spectra and its narrowness means that burning affects the first purely electronic zero-phonon transition rather than the vibronic or higher electronic ones [18,19]. Thus we came to the conclusion that in leaves at low temperatures Chl *a* forms with S_1-S_0 energies ranging from 13400 cm^{-1} (745 nm) to 14300 cm^{-1} (700 nm) are represented.

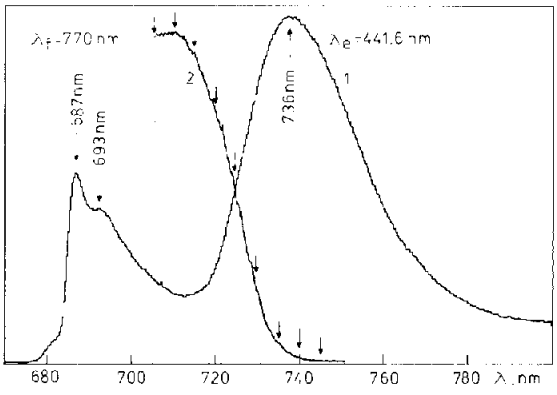

Fig.1. Fluorescence spectrum (curve 1) (excited at 441.6 nm) and excitation spectrum (curve 2) (recorded at 770 nm) of a greening maize leaf at 5 K. Holes were burnt at λ denoted by arrows.

The saturated deepness of holes revealed that the contribution of zero-phonon lines to the red-shifted $S_1 \longleftarrow S_0$ and $S_1 \longrightarrow S_0$ transition intensities of Chl *a* in plants is not essentially lower than in glassy diluted solutions of the same pigment [11].

'Selective' excitation in the 700–745 nm region produces a broad fluorescence band with no resolved vibronic structure (fig.3, curve 2). In contrast to the selective fluorescence spectra of monomeric Chl *a*, where the intensity maximum lies close to λ_e and corresponds to the phonon wing of the 0-0 transition and to the pseudo-wings of the nearby-positioned 0-0 lines [11], the broad maximum in leaves is remarkably red-shifted (by 15–25 nm). The red-shift of the fluorescence band and weakening of lines have been observed in con-

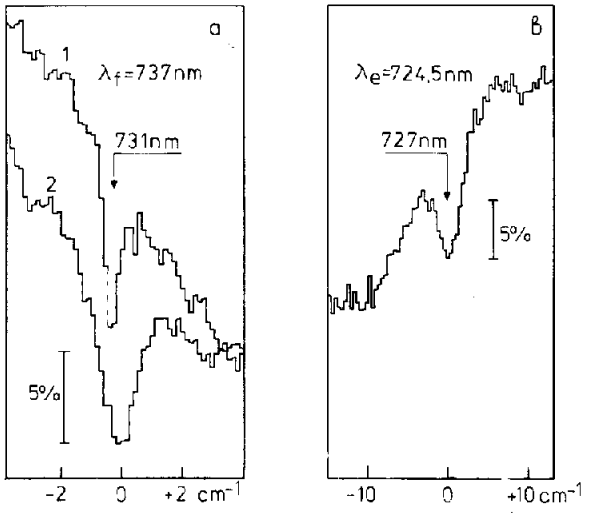

Fig.2. Holes in excitation (curve 1) and fluorescence (curve 2) spectra, burnt with a single-frequency (δ < 0.003 nm) (a) and a broad-band (δ = 0.03 nm) (b) laser, respectively (intensity, 4 mW·cm^{-2}; exposure time, 4 min). Excitation spectra were obtained by scanning the broad-band laser with broad-band fluorescence recording (1 nm).

centrated (10^{-2} M) monomeric Chl *a* solutions in frozen organic glasses where energy migration takes place [20]. The energy transfer leads to an effective disappearance of vibronic lines simply because the absorption and emission acts are located at different molecules with different S_1-S_0 energies.

As far as the vibronic band intensity (at 810 nm) in fluorescence is concerned (fig.3, curve 2), it comprises about 10% of the 0-0 band as in the case of the 'free' pigment. Furthermore, the distance between 0-0 and vibronic bands (~1250 cm^{-1}) remains the same as in Chl *a* solutions.

The nature of absorption in the long-wavelength region in leaves is less clear. It is highly probable that the higher excitonic components of the $S_1 \longleftarrow S_0$ (and $S_2 \longleftarrow S_0$) transition make the main contribution to the absorption cross-section. This explains why the holes in absorption are so shallow. The level-splitting has been documented for Chl-pigment complexes absorbing at $\lambda < 700$ nm by circular dichroism measurements [17,21], but to our knowledge no definite evidence is available about the excitonic interactions in the spectral range $\lambda >$ 700 nm.

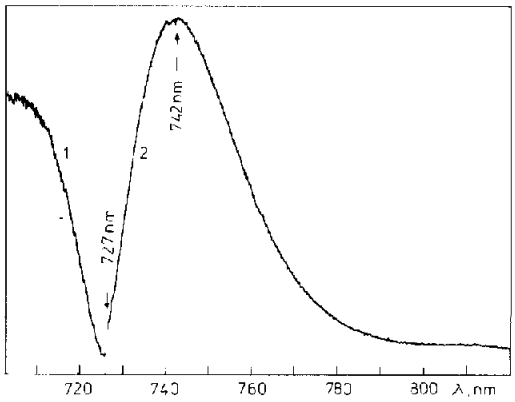

Fig.3. Selective fluorescence (curve 1) and excitation (curve 2) spectra of a greening maize leaf. Excitation (recording) was set in the region of purely electronic zero-phonon transitions of the long-wavelength forms of Chl *a* at 727 nm.

Summarizing the results presented above, we found that at 5 K the 736 nm fluorescence band of plants is built up largely by purely electronic zero-phonon transitions and their phonon wings. The large Stokes shift between fluorescence and excitation contours (fig.1, curve 3) is conditioned, first, by an efficient directed energy transfer within an inhomogeneously broadened S_1-S_0 manifold of centers and, second, by absorption via higher excitonic levels, rather than by the enhancement of vibronic transition intensities at the expense of 0-0 ones, as stated earlier [10]. The relative concentration of fluorescent traps with S_1-S_0 energy at 730–740 nm is quite low, although the latter receive most of the energy even when excited at λ_e < 700 nm. At physiological temperatures these traps cannot compete with reaction centers due to thermally-activated energy back-transfer processes [22]. It is, however, plausible, that these forms are further red-shifted at low temperature, owing to the contraction of the protein matrix, which brings pigment molecules closer to one another.

Strong pigment-pigment and/or pigment-protein interactions capable of shifting the transition energies by $\geq 10^3$ cm^{-1} should by no means be accompanied by any remarkable strengthening of electron-phonon coupling. It has been shown that in the photoactive protochlorophyllide P-656 in etiolated leaves, whose fluorescence maximum is red-shifted by 30 nm in comparison with solutions of free pigment, deep holes can be formed and

high-resolution fluorescence spectra obtained [14].

Further studies of temperature and wavelength dependence of the hole-widths should establish whether any relaxation time (fluorescence decay or photon echo decay times [23]) is correlated with the linewidths of persistent spectral holes.

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REFERENCES

[1] Seely, G.R. (1977) in: Primary Processes of Photosynthesis (Barber, J. ed.) pp.1–53, Elsevier, Amsterdam.
[2] Katz, J.J., Shipman, L.L., Cotton, T.M. and Janson, T.R. (1978) in: The Porphyrins (Dolphin, D. ed.) vol.V, pp.401–458, Academic Press, New York.
[3] Pearlstein, R. (1982) in: Photosynthesis. Energy Conversion by Plants and Bacteria (Govindjee ed.) pp.295–330, Academic Press, New York.
[4] Scherz, A. and Parson, W.W. (1984) Biochim. Biophys. Acta 766, 666–678.
[5] Eccles, J. and Honig, B. (1983) Proc. Natl. Acad. Sci. USA 80, 4959–4962.
[6] Mullet, J.E., Burke, J.J. and Arntzen, C.J. (1980) Plant Physiol. 65, 814–822.
[7] Satoh, K. and Butler, W.L. (1978) Plant Physiol. 61, 373–379.
[8] Rijgersberg, C.P., Amesz, J., Thielen, A.P.G.M. and Swager, J.A. (1979) Biochim. Biophys. Acta 545, 473–482.
[9] Machold, O., Simpson, D.J. and Moller, B.L. (1979) Carlsberg Res. Commun. 44, 235–254.
[10] Kochubei, S.M. (1986) The Pigment Organization of Photosynthetic Membranes as a Basis of Energy Supply in Photosynthesis, p.89, Naukova Dumka, Kiev (in Russian).
[11] Avarmaa, R.A. and Rebane, K.K. (1985) Spectrochim. Acta 41A, 1365–1380.
[12] Friedrich, J., Scheer, H., Zickendraht-Wendelstadt, B. and Haarer, D. (1981) J. Chem. Phys. 74, 2260–2266.
[13] Maslov, V.G., Chunaev, A.S. and Tugarinov, V.V. (1981) Mol. Biol. 15, 1016–1027.
[14] Renge, I., Mauring, K. and Avarmaa, R. (1984) Biochim. Biophys. Acta 766, 501–504.
[15] Gillie, J.K., Fearey, B.L., Hayes, J.M., Small, G.J. and Golbeck, J.H. (1987) Chem. Phys. Lett., in press.
[16] Strasser, R.J. and Butler, W.L. (1977) Biochim. Biophys. Acta 462, 307–313; Mohanty, P., Zilinskas Brown, B., Govindjee and Thornber, J.P. (1972) Plant and Cell Physiol. 13, 81–91; Boardman, N.K., Thorne, S.W. and Anderson, J.M. (1966) Proc. Natl. Acad. Sci. USA 56, 586–593.
[17] Gulyaev, B.A. and Teten'kin, V.L. (1983) Izv. Akad. Nauk SSSR, Ser. Biol. 536–552.
[18] Rebane, K.K. (1970) Impurity Spectra of Solids, Plenum, New York.
[19] Rebane, L.A., Gorokhovskii, A.A. and Kikas, J.V. (1982) Appl. Phys. B29, 235–250.
[20] Avarmaa, R., Jaaniso, R., Mauring, K., Renge, I. and Tamkivi, R. (1986) Mol. Phys. 57, 605–621.
[21] Gregory, R.P.F. (1977) in: Primary Processes in Photosynthesis (Barber, J. ed.) pp.465–492, Elsevier, Amsterdam.
[22] Butler, W.L., Tredwell, C.J., Malkin, R. and Barber, J. (1979) Biochim. Biophys. Acta 545, 309–315.
[23] Meech, S.R., Hoff, A.J. and Wiersma, D.A. (1985) Chem. Phys. Lett. 121, 287–292.