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RADIATIVE AND NONRADIATIVE TRANSITIONS IN SUBCHLOROPLAST PARTICLES HIGHLY ENRICHED IN *P*-700

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Summary

Radiative and nonradiative processes were investigated in subchloroplast particles highly enriched in *P*-700 (1 *P*-700 to 10 chlorophyll molecules) according to the method of classical fluorescence and of photoacoustical spectroscopy. The envelope of fluorescence spectrum divided into three Gaussian bands and their quantum yields of fluorescence were calculated. Independently the quantum yield of fluorescence was determined from the spectral course of the photoacoustical signal. Finally, the estimate of the photochemical activity of *P*-700, based upon the measured fluorescence quantum yield and upon the measured nonradiative losses of excitation energy, was done.

Introduction

In recent papers [1—4] the optical and steady-state fluorescence properties of subchloroplast particles enriched in *P*-700 have been measured by means of optical spectroscopy. Ikegami et al. [5] has observed the variable fluorescence in the same particles and Shuvalov [6] has measured on the basis of similar particles the luminescence kinetic. The understanding or primary processes of photosynthesis, however, requires a knowledge of primary nonradiative processes occuring in *P*-700 energy levels determined from the spectroscopical measurements. For this reason, the absolute fluorescence quantum yield of subchloroplast particles enriched in *P*-700 was determined using the methods of classical fluorescence and photoacoustic spectroscopy *.

* See footnote on p. 342.

Materials and Methods

Materials

All measurements presented were made in subchloroplast particles highly-enriched in *P*-700 (10 chlorophyll *a* molecules to 1 *P*-700) and in a model system containing the mixture of chlorophyll *a* and bovine serum albumin coagulated in acetone/water solutions (analytical grade) at room temperature; the isolation method of the *P*-700-enriched particles starting from spinach leaves and the preparation of the model system have been described elsewhere [1,8].

Rhodamine B (Lamba Physik, Göttingen, F.R.G., laser dye grade) was used as the fluorescence standard. After the analysis of published data had been done (e.g. [9,10]), a value of 0.7 was chosen as the quantum fluorescence yield of rhodamine B in basic ethanol solution (10^{-4} M). Quantum yield of rhodamine B in powder form $\eta = 0.53$ was absolutely determined from photo-acoustical measurements.

Methods

Fig. 1 shows the experimental diagram of the apparatus used. The illumination system consisted of a 100 W xenon lamp Narva XBO 101, a Jobin-Yvon H 10 monochromator (dispersion 8 nm/mm), and a home-made fixed frequency (25 Hz) mechanical light chopper. The fluorescence detection unit consisted of a Carl Zeiss SPM 2 grating monochromator (dispersion 4 nm/mm) equipped with photomultiplier RCA 7102 or quantacon C 31034, which operated as a photon counter or was connected with a lock-in amplifier. The recorded fluorescence signal was corrected for spectral sensitivity of photomultiplier photocathode.

The sample cell was made from a polished cylindrical block of plexiglass. The metal sample holder and microphone mount were readily attached to the plexiglass with O-ring seals. A thin layer of powdered sample was spread onto a quartz plate at the end of the sample holder. The excitation light was focused vertically downward into the sample cell through the plexiglass enabling powder and liquid samples to be handled as easily as solid materials. The cell volume ($\sim$0.3 cm^3) consisted of the volume of the cylindrical hole in the plexiglass block not occupied by the sample holder. The measuring chamber with a microphone enabled simultaneous fluorescence and photoacoustical signal recording.

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* Photoacoustic spectroscopy is a recently revived technique that enables spectra, correlated under certain conditions [7] to optical absorption spectra, to be obtained on any type of solid or liquid. Thus, photoacoustic spectroscopy offers special advantages when opaque and/or highly scattering samples are to be studied. Periodically interrupted light absorbed by the sample is converted by radiationless processes to heat, which partially flows from the sample surface to gas in the sample cell. If the gas volume is enclosed and has a sensitive microphone in it, pressure waves detectable as sound vibrations can be obtained using phase-sensitive detection. Photoacoustic spectroscopy has been used to study the optical and thermal properties of solids and solutions and for the study of energy transfer processes [7]. It is applicable to the study of these processes since photoacoustic spectroscopy signals are produced only by the absorbed light actually converted to heat in the sample. Absorbed light that is reradiated as luminescence, or trapped as stored energy does not contribute to the photoacoustic spectroscopy signal.

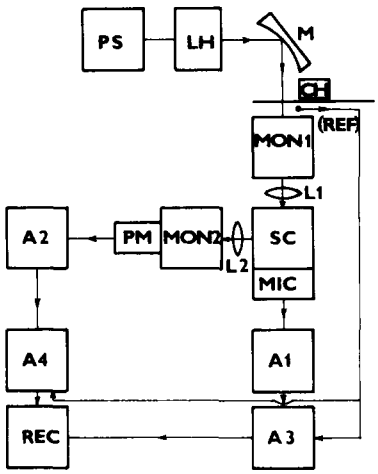

Fig. 1. Experimental arrangement. PS, stabilized power supply VEB Wetron; LH, lamp housing with Narva XBO 101 xenon lamp; M, spherical reflector $f = 100$; MON 1, Jobin-Yvon H 10 monochromator with motor drive; SC, sample cell; MIC, 1/2" GenRad microphone Model 1962-9602; CH, light chopper; L1/L2 glass lenses $f = 25$; MON 2, Carl Zeiss SPM 2 monochromator with motor drive; PM, photomultiplier (RCA 7102 or Quantacon C 31034); A1/A2, preamplifiers (Al GenRad Model 1972-9600; A2 Unipan Model 233.5); A3/A4, Unipan lock-in Model 233; REC, chart recorder Servogor Model RE 511.

The pressure oscillation in the cell gas (air) was detected by a General Radio electret microphone Model 1962-9602 followed by General Radio preamplifier Model 1972-9600. The preamplifier signal was measured by lock-in amplifier Unipan Model 233. Correction of the photoacoustic spectroscopy spectra for variation of excitation light intensity with wavelengths was achieved by dividing the sample spectrum by the carbon black photoacoustic spectroscopy spectrum.

Absolute fluorescence quantum yield of the *P*-700-enriched subchloroplast particles was fixed for the excitation wavelength $\lambda_{exc} = 430$ nm as follows: The envelope of fluorescence spectrum was separated into Gaussian bands, and to eliminate the spectrum background we used the polynom * as a mathematical filter. The calculated parameters of corresponding bands (see Table I) were used for an accurate determination of the integrated intensities which were calibrated by fluorescence standard of rhodamine B in powder. The use of rhodamine B in powder form was stimulated by the effort of maximal approach to the measured character of our measured samples. The quantum yield of chlorophyll *a* was similarly determined from comparison of the standard of rhodamine B in ethanol solution.

Independently, fluorescence quantum yield was determined from the course of the photoacoustical signal. The fluorescence quantum yield can be calculated as

$$\eta = 1 - P_{\lambda}^{AS}/P_{0\lambda}(\aleph, x)$$

where $P_{0\lambda}$ represents the light power absorbed in the sample at wavelength λ,

* The parameters of separated bands were fitted according to the method of dumped least squares [11]. The attained convergence enabled a calculation of standard deviations of obtained parameters.

TABLE I

FLUORESCENCE SPECTRUM DECONVOLUTION OF SUBCHLOROPLAST PARTICLES ENRICHED IN *P*-700 INTO GAUSSIAN BANDS

(A) separation of the fluorescence spectrum published in [1]; (B) separation of the fluorescence spectrum in Fig. 2b.

Band	Band maximum λ_{max} [nm]		Band intensity A_{max} [arbitrary units]		Band halfwidth $\Delta\gamma_{1/2}$ [cm^{-1}]	
	A	B	A	B	A	B
1	673.3 ± 0.2	676.6 ± 0.3	69.0	77.6	809 ± 11	769 ± 14
2	703.0 ± 0.2	703.2 ± 0.4	19.0	26.9	340 ± 15	426 ± 23
3	730.0 ± 0.5	732.6 ± 0.5	72.3	63.7	1367 ± 25	1027 ± 21

P_λ^{AS} is the photoacoustical power (mainly determined by nonradiative transitions) of the signal at wavelength λ, $\aleph$ is the absorption coefficient and x is the sample width.

Results

Typical fluorescence and photoacoustic spectra of the *P*-700-enriched subchloroplast particles and of the model system are shown in Fig. 2a and b. In both cases the fluorescence spectrum consists of three bands and qualitatively resembles the fluorescence spectrum of chlorophyll *a*, the photoacoustic spectrum then respects absorption course of chlorophyll *a*. Compared to chlorophyll *a* solution or in polymer matrix fluorescence spectra [12] the long wavelength fluorescence band at 730 nm is more intensive.

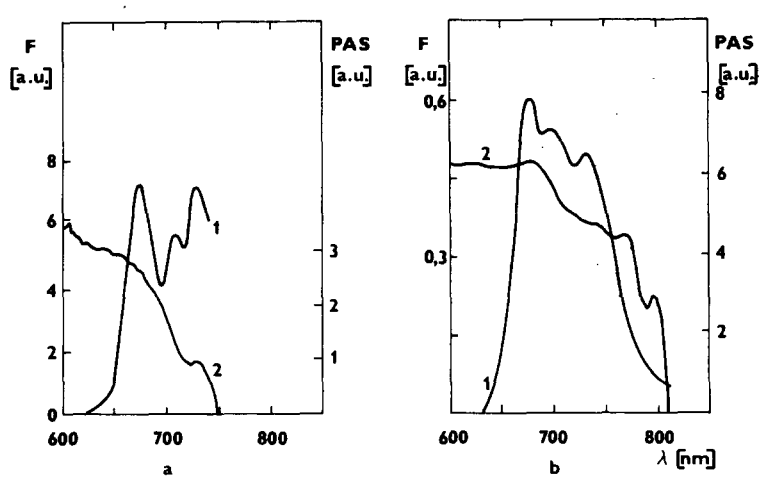

Fig. 2. (a) Fluorescence spectrum (1) and photoacoustic spectrum (2) of the model system containing chlorophyll *a* (6 μM) and bovine serum albumin (30 μM) in water/acetone solution (1 : 2) at 20°C. (b) Fluorescence spectrum (1) and photoacoustic spectrum (2) of subchloroplast particles enriched in *P*-700 at 20°C. (F scale corresponds to the fluorescence (maximum value ~$0.1 \cdot 10^{-7}$ W), photoacoustic spectroscopy corresponds to photoacoustical signal (maximum value ~$0.7 \cdot 10^{-6}$ W), photoacoustic spectroscopy spectra have been normalized to carbon black.)

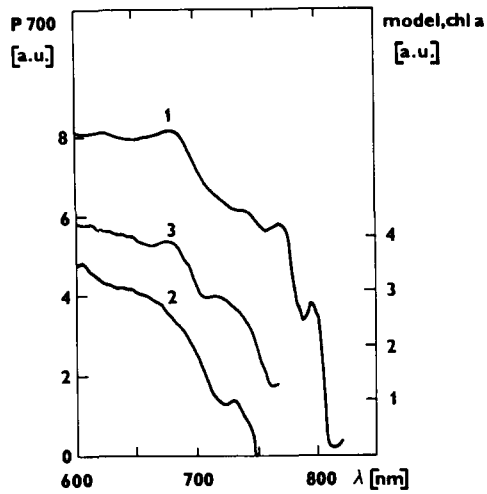

Fig. 3. The course of photoacoustic signal of chlorophyll *a* (curve 3) of model system (chlorophyll *a* (6 μM) and bovine serum albumin (30 μM)) in powder (curve 2) and of subchloroplast particles enriched in *P*-700 in powder (curve 1) at 20°C. The left scale is for subchloroplast particles, the right scale for the curves 2 and 3. The value for λ = 675 nm on the curve 3 corresponds approx. to the energy $0.3 \cdot 10^{-6}$ W. All spectra have been normalized to carbon black.

Fig. 3 shows a typical course of photoacoustical signal of chlorophyll *a*, of model system (chlorophyll *a* and bovine serum albumin) and of *P*-700-enriched subchloroplast particles; all three samples were in powder form. The spectrum of subchloroplast particles and of model systems again qualitatively resembles the course of chlorophyll *a*, an important difference only appears in subchloroplast particles in the long wavelength spectral region ($\lambda > 750$ nm).

Summary of the calculated quantum yields of the fluorescence for *P*-700-enriched subchloroplast particles and for chlorophyll *a* are given in Table II.

Let us concern first with the fluorescence spectrum of subchloroplast particles enriched in *P*-700. To compare our present results we separated the fluorescence spectrum of these particles (previously published in Ref. 1) into Gaussian bands. In all cases the separation led to three bands, the results are presented in Table I. It shows that the fluorescence of subchloroplast particles highly enriched in *P*-700 is characterized by three bands: at 673 (676) nm, at

TABLE II

FLUORESCENCE QUANTUM YIELD (η)

The experimental error in the quantum yield determination is estimated to 50%. It is mainly done by a different character of the sample and the standard (including fluorescence reabsorption).

System	η determined from fluorescence measurements (excitation at 430 nm)	from photoacoustic spectroscopy
Chlorophyll *a*	0.21	0.20
Subchloroplast particles:		
band at 675 nm	0.012	0.017
band at 703 nm	0.005	
band at 730 nm	0.020	

703 nm and at 730 (732) nm. The dispersion of fluorescence band values is probably due to the difference of plant material used in both laboratories. In the photoacoustic spectrum the first and the third fluorescence bands have corresponding maxima for the model system and for subchloroplast particles enriched in *P*-700; this is similar to chlorophyll *a* (see Fig. 3, curve 3); subchloroplast particles have in addition two pronounced bands in the long wavelength region of the photoacoustical spectrum, at 770 and at 795 nm, and the pronounced minimum at 810 nm. The origin of these bands could be probably connected with the protein content in *P*-700; our photoacoustic spectroscopy measurements on the model system with bovine serum albumin did not give any evidence for this hypothesis.

Discussion

Let us start our discussion by considering the calculated values of fluorescence quantum yield, determined from fluorescence or photoacoustic measurements. First of all, a quite reasonable correspondence of the yield values obtained by two different methods may be seen. The fluorescence quantum yield is almost the same for the bands at 675 and 730 nm (see Table II). About 3.5% of the total light energy absorbed by subchloroplast particles is emitted in all three fluorescence bands, that means that 96.5% of the absorbed light energy is transformed into another king of energy (mostly heat).

Analysing the photoacoustic spectrum of subchloroplast particles enriched in *P*-700 we may conclude that the following dissipation of the absorbed light energy into heat appears in the corresponding fluorescence bands: for the band at 675 nm 46%, for the band at 703 nm 36%, and finally for the band at 730 nm 18% only.

We can see from this analysis that the centre, responsible for the fluorescence band at 730 nm, has the least heat dissipation of the electron excitation energy of all three measured bands (in fact it is twice less than the remaining two centres responsible for fluorescence bands at 675 and 703 nm). It is usually considered that *P*-700 is more or less responsible for the fluorescence band at 730 nm. To progress further we can take the knowledge of the fluorescence quantum yield for the 732 nm band into account as the amount of electron excitation energy absorbed (or transfered to) by this centre and if we add the electromagnetic energy emitted in the 732 nm band (determined from fluorescence quantum yield) to the energy dissipated to heat by this centre (determined from the photoacoustic spectroscopy curve), we can speculate about the energy portion which can only be realized in photochemical activity of the centre. In our case this portion amounts to about 80% of the absorbed energy for the 732 nm band. This value is quite narrow compared with the estimate by Borisov [13].

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