

Fluorescence and Photochemical Properties of Plants with Defective Photosystem II

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The mykotrophic orchid *Neottia nidus-avis* does not evolve oxygen in the light but is able to perform photophosphorylation. The low temperature fluorescence emission spectrum lacks the 680 and 690 nm bands. Hence, the spectroscopic chlorophyll a forms which are attributed to photosystem II do not occur in plastids of this orchid. The low temperature excitation spectrum of photosystem I fluorescence exhibits a maximum at 666 nm. The position of this maximum appears not to be influenced by energy transfer and corresponds to the absorption maximum of the chlorophyll form which emits the photosystem I fluorescence. Energy migration, however, occurs from carotenoids whose absorption spectrum is shifted to longer wavelengths and which cause the yellow-brown color of the *Neottia* plastids. Room temperature fluorescence emission shows after the onset of light no variable part.

Despite the fact that plastids of the *tobacco* mutant *NC 95* at most evolve only traces of oxygen the low temperature emission spectrum shows the three bands which are usually observed with fully functioning chloroplasts. However, the two bands at 680 and 690 nm are distinctly lower than with the wild type. The variable portion of room temperature fluorescence is barely detectable. In line with the very low capacity for oxygen evolution, rates of electron transport partial reactions in the region of photosystem II are extremely low. In agreement with this observation no 690 nm absorption change signal is detected. However, a normal P_{700}^{+} signal is seen. In the presence of electron donors like reduced phenazine methosulfate the decay time of the P_{700}^{+} signal is faster than with the wild type.

The yellow *tobacco* mutant *Su/su* var. *aurea* which exhibits at high light intensities higher rates of photosynthesis than the wild type shows at low temperature an emission spectrum with stronger photosystem II bands than the wild type.

Plastids of the mykotrophic orchid *Neottia nidus-avis* do not evolve oxygen in the light. In chloroplasts of the *tobacco* mutant *NC 95* photosystem II is also defective. The chloroplasts of this *tobacco* mutant evolve, however, traces of oxygen. On other hand both plants are able to perform photophosphorylation [1, 2]. The spindle shaped plastids of *Neottia* contain rolled-up thylakoids, which have just as the thylakoids of the *tobacco* mutant no touching areas [3–6]. *Neottia* contains no chlorophyll b [7]. The

plastids are yellow-brown which is caused as in *Phaeophyceae* by the fact that carotenoid absorption is shifted to longer wavelengths. This has been attributed earlier already to binding of carotenoids onto proteins [8].

In the present paper we attempt to characterize the plastid defects concerning photosystem II by means of fluorescence and photochemical studies. For comparison purpose we used the *tobacco* wild type and the *tobacco* mutant *Su/su* var. *aurea*.

Abbreviations: DCMU, N-(3,4-dichlorophenyl)-N,N'-dimethylurea; PMS, phenazine methosulfate.

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Materials and Methods

Plants material

The variegated *tobacco* mutant *NC 95* and the mutant *Su/su* var. *aurea* were grown in the green-



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house. Green leaf patches of the variegated mutant *NC 95* served as a control. *Neottia nidus-avis* was field-grown.

For the low temperature spectra leaf patches were dipped in dim light into liquid nitrogen and ground with ice. The preparations were filled into cooled quartz tubes (diameter 3 mm) or in quartz cuvetts (path length 1 mm). The maximal absorption per cm was for fluorescence smaller than 0.1. For the base line the tubes were filled with powdered ice.

Spectroscopy

The low temperature absorption spectra were taken with a Cary-Spectrophotometer 14R, equipped with a scattered transmission accessory, model 1462400. The cuvetts were cooled with liquid nitrogen in a special holder. Temperature was measured in the cuvet with a copper-constantan thermocouple. The sample space was sparged with dried and cooled nitrogen. Registration was carried out at 118 °K.

The low temperature fluorescence spectra were made with a Farrand MK-1 Spectrofluorometer, equipped with an excitation correction and an emission correction module. The control and adjustment of the emission correction was carried out according to ref. [9] with a tungsten calibration lamp (Osram-Glühlampe für wissenschaftliche Zwecke Typ Wi41/G). The electrical data were determined in the Physikalisch-Technische Bundesanstalt, Braunschweig. The current was taken from a stabilized power supply (Heinzinger TN 5650). The correction of the excitation spectra was checked with a diluted solution of chlorophyll *a* in acetone. After a new adjustment of the excitation module by the producer firm the excitation spectrum of chlorophyll *a* was by maximally 5 per cent different from the absorption spectrum. The wavelength scales were checked with neon and mercury spectral calibration sources (Ultraviolet Products, Ind.), since during the base line registration considerable drift phenomena were observed, the photomultiplier was darkened in short time intervals and the dark current corrected to zero. Light scattering was diminished by suitable cut-off and interference filters. Distortions of the spectra, caused by optical inhomogeneities of the preparations and by high local pigment concentrations in the thylakoids were not corrected [10]. Therefore, the

spectra are not usable for the calculation of quantum yields.

Registration of *fluorescence induction curves* was carried out as described earlier [11]. The leaf patches formed with the exciting beam an angle of 45 °.

The *resolution of the spectra* was carried out with a Dupont Curve Resolver 310. The two short wavelength bands of the low temperature emission spectrum were approximated by a normal distribution each, whereas for the approximation of the longer wavelength band (~ 730 nm) two normal distributions were necessary.

Electron transport reactions were carried out as described earlier [12].

Flash light spectroscopy was carried out according to the literature [13] with a rhodamine dye laser by measuring the absorption change induced by a single flash at 820 nm.

Results and Discussion

At liquid nitrogen temperatures chlorophyll *a* of the two photosystems fluoresces at different wavelengths. According to Butler and Kitajima [14] the antenna chlorophyll of photosystem I emits at approximately 730 nm and the antenna chlorophyll of photosystem II at around 690 nm. The band at 680 nm is ascribed to the light-harvesting chlorophyll. Due to energy migration the intensity of this fluorescence band depends on photosystem II. Therefore, it can be assumed that the number of quanta which is emitted at the mentioned wavelengths depends on the number of fluorescing chlorophyll *a* molecules in the two photosystems. In addition, this number is influenced by the migration of excitation energy from photosystem II to photosystem I which in turn depends on the state of thylakoid membrane [15, 16; for further references 17, 18]. For the conclusions reached in our paper the latter influences are not relevant.

The emission spectrum shown in Fig. 1a demonstrates that in *Neottia* no emission is observed at wavelengths characteristic for photosystem II. In comparison to this the spectrum of the *tobacco* mutant *NC 95* clearly shows these bands (Fig. 1b) which, however, are a distinctly lower than in the *wild type* (Fig. 1d). In the *tobacco aurea* mutant, on the other hand the photosystem II dependent emission is distinctly higher than in the *wild type* (Fig. 1c). After resolution of the emission spectra into

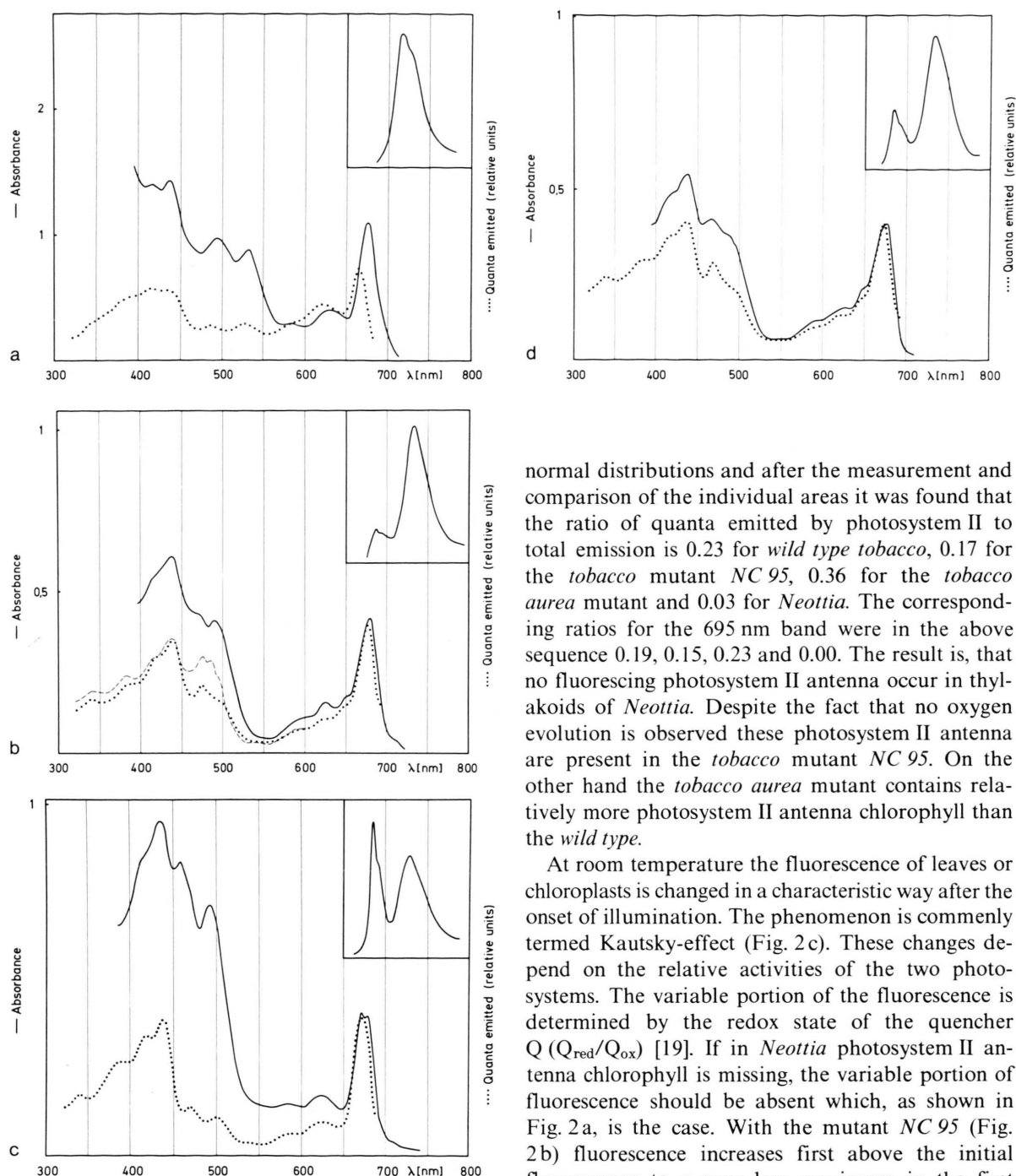


Fig. 1. Low temperature absorption spectra—, low temperature excitation spectra of the emission at 730 nm ·····, and excitation spectrum of the emission at 683 nm ----. Inset: Low temperature emission spectra—, a) *Neottia nidus-avis*. b) Yellow-green leaf section of *Nicotiana tabacum* NC 95. c) *Nicotiana tabacum* Su/su var. aurea. d) *Nicotiana tabacum* wild type.

normal distributions and after the measurement and comparison of the individual areas it was found that the ratio of quanta emitted by photosystem II to total emission is 0.23 for *wild type tobacco*, 0.17 for the *tobacco* mutant NC 95, 0.36 for the *tobacco aurea* mutant and 0.03 for *Neottia*. The corresponding ratios for the 695 nm band were in the above sequence 0.19, 0.15, 0.23 and 0.00. The result is, that no fluorescing photosystem II antenna occur in thylakoids of *Neottia*. Despite the fact that no oxygen evolution is observed these photosystem II antenna are present in the *tobacco* mutant NC 95. On the other hand the *tobacco aurea* mutant contains relatively more photosystem II antenna chlorophyll than the *wild type*.

At room temperature the fluorescence of leaves or chloroplasts is changed in a characteristic way after the onset of illumination. The phenomenon is commonly termed Kautsky-effect (Fig. 2c). These changes depend on the relative activities of the two photosystems. The variable portion of the fluorescence is determined by the redox state of the quencher Q (Q_{red}/Q_{ox}) [19]. If in *Neottia* photosystem II antenna chlorophyll is missing, the variable portion of fluorescence should be absent which, as shown in Fig. 2a, is the case. With the mutant NC 95 (Fig. 2b) fluorescence increases first above the initial fluorescence to a very low maximum in the first 1/10 second, but thereafter falls slowly back to the initial value. The slow fluorescence decrease is attributed by several authors to changes in the thylakoid membrane condition which in turn should be connected to the built-up of the proton gradient [18].

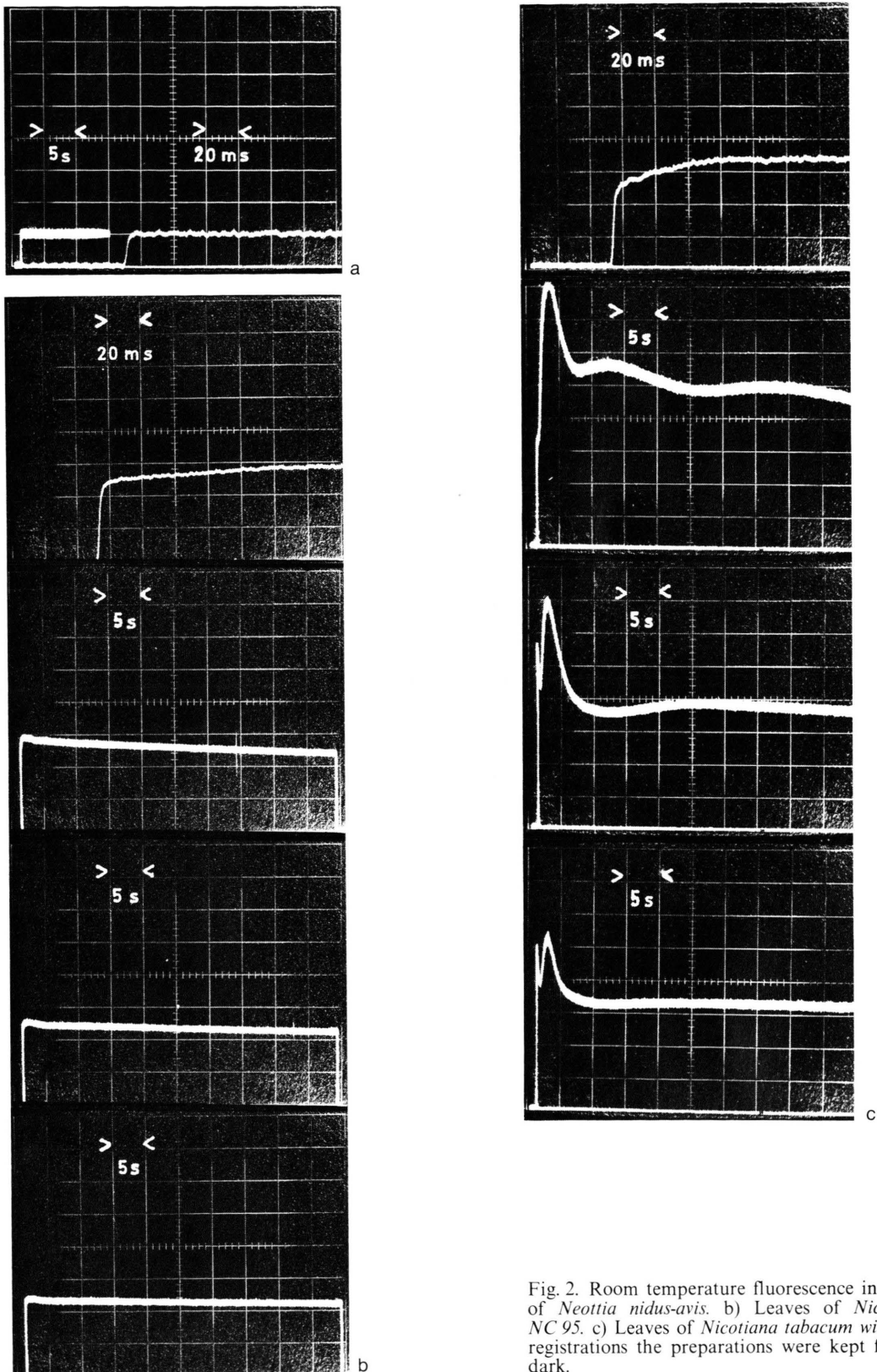


Fig. 2. Room temperature fluorescence induction. a) Lips of *Neottia nidus-avis*. b) Leaves of *Nicotiana tabacum* NC 95. c) Leaves of *Nicotiana tabacum* wild type. Between registrations the preparations were kept for 1 min in the dark.

Above all French and co-workers [20] have shown that different spectroscopic forms of chlorophyll *a* exist in chloroplasts. By means of the low temperature excitation spectra shown in Fig. 1 one can evaluate which one of these chlorophyll forms excites the emission band at 730 nm. Already the comparison of the fluorescence excitation spectra with the corresponding absorption spectra shows the red maximum of the excitation spectra at somewhat shorter wavelengths than the red maximum of the absorption spectrum [21]. In detail it is seen that with *Neottia* the maximum of the excitation spectrum lies at shorter wavelengths when compared to *tobacco*. On the other hand the absorption maximum of *Neottia* falls into the region in which the *tobacco* plants exhibit their absorption maxima. Moreover, the fluorescing portion of chlorophyll seems to be especially low with *Neottia*. This is the reason why the fluorescence curve crosses the absorption curve. At 666 nm only a portion of the chlorophyll absorbing at that wavelength fluoresces. The position of the maxima is verified by Table I. It is noteworthy, that at low temperature the absorption maximum of the *aurea* mutant splits (Fig. 1c). In the mutant *NC 95* it is shown that absorption between 450 and 500 nm increases the 685 nm fluorescence emission stronger than that of the 730 nm band (Fig. 1b). This was to be expected with the mutant *NC 95* if the pigments of the photosystem II antenna were still present. Hence, energy transfer from carotenoids to the pho-

tosystem II antennae still functions in this mutant. Concerning *Neottia*, the comparison between room temperature excitation and absorption spectra did not give the information whether the carotenoid absorption, shifted to the green (540 nm), contributes to fluorescence [1]. However, the comparison of the low temperature spectra shows that this is the case (Fig. 1a). The question remains, at what wavelength in intact chloroplasts the absorption maximum of the chlorophyll *a* form is situated which emits at around 730 nm. This cannot be directly deduced from the fluorescence excitation spectra, which is due to energy transfer from photosystem II to photosystem I. As the plant does not contain photosystem II pigments, this energy migration cannot occur in *Neottia*. Therefore, we may assume that the red band of the excitation spectrum corresponds in *Neottia* to the absorption band of the chlorophyll form which emits photosystem I fluorescence. There, energy transfer of the bulk chlorophyll can apparently not occur or only to a small extent because of its absorption maximum at longer wavelengths. It is expected that this chlorophyll form absorbs at longer wavelength than *Neottia* in intact chloroplasts, since the emission maxima are situated not at 719 nm but at 730–735 nm.

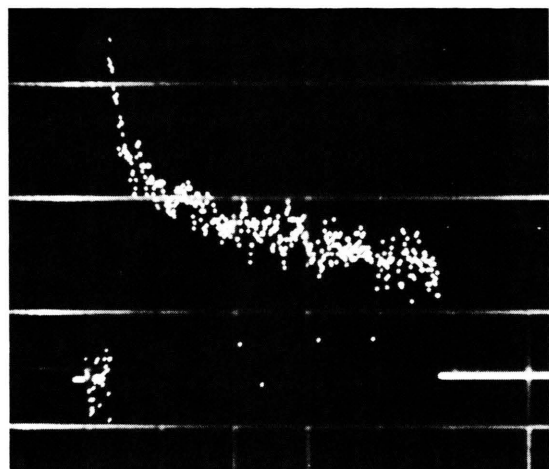
The inhibition of oxygen evolution in the *tobacco* mutant *NC 95* is primarily not due to the lack of photosystem II pigments as is the case with *Neottia* but must have other reasons. In order to characterize this defect better we have checked partial

Table I. Position of the red maxima of the low temperature spectra.

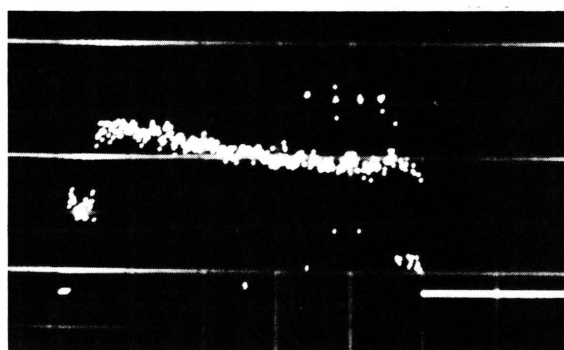
	Position of absorption maximum	Maximum of the fluorescence excitation spectrum for the 730 nm emission band	Position of emission maximum
<i>Nicotiana tabacum</i> , wild type	675 nm	673 nm	733 nm
<i>Nicotiana tabacum</i> , mutant <i>NC 95</i>	678 nm	675 nm	734 nm
<i>Nicotiana tabacum</i> <i>Su/su</i> var. <i>aurea</i>	672, 678 nm	671 nm	730 nm
<i>Neottia nidus-avis</i>	677 nm	666 nm	719 nm

Table II. Anthraquinone-2-sulfonate-reduction in chloroplasts from yellow leaf patches of variegated *N. tabacum* var. *NC 95*.

Donor System	μmol acceptor reduced $\cdot (\text{mg chlorophyll})^{-1} \cdot \text{h}^{-1}$
2,6-Dichlorophenol indophenol/ascorbate	3673
Tetramethyl benzidine/ascorbate	54
Diphenylcarbazide 5×10^{-3} M	0
Diphenylcarbazide 1.8×10^{-2} M	56
Diphenylcarbazide 1.8×10^{-2} M + DCMU	42
Diphenylcarbazide 1.8×10^{-1} M	127
Diphenylcarbazide 1.8×10^{-1} M + DCMU	116



a



b

Fig. 3. Amplitude of the absorption change at 820 nm in chloroplasts induced by a single rhodamine dye laser flash. a) *Nicotiana tabacum* NC 95. b) *Nicotiana tabacum* wild type. Time scale: 200 μ sec/division, a) 30 μ g Chlorophyll/3 ml assay; absorption change $\Delta A = 0.25 \times 10^{-4}$ per division b) 40 μ g Chlorophyll/3 ml assay; absorption change $\Delta A = 0.625 \times 10^{-4}$ per division.

reactions of electron transport in the region of photosystem II. From Table II it is seen that tetramethyl benzidine or diphenylcarbazide, which are artificial electron donors to photosystem II, mediate only extremely low reaction rates. Moreover, it should be noted that with high diphenyl-carbazide concentrations in the assay a DCMU insensitive and consequently a photosystem I reaction is induced [12]. Reasonable electron transport rates occur only in the region of light reaction I such as in the anthraquinone-2-sulfonate photoreduction with the electron donor couple dichlorophenol indophenol/ascorbate. This incapability of chloroplasts to perform photosystem II reactions is apparently due to the absence of intact photosystem II reaction centers. By single flash spectroscopy we were able to show that the observed absorption change signal at ~ 820 nm is entirely due to photosystem I. As was expected from the present studies the reaction center of photosystem I is fully developed, and the absorption change signal attributed to photosystem I (P_{700}^+) is at least of the same size as that of the *wild type* when chloroplasts corresponding to comparable amounts of chlorophyll are tested (Fig. 3a and b). According to the literature an 810 nm absorption change is attributed to P_{700}^+ [13]. As seen in Fig. 3a in the presence of phenazine methosulfate the relaxation kinetics are faster in the mutant NC 95 when compared to that of the *wild type* (Fig. 3b).

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