On the Correlation between the Amplitude of the Electrochromic Absorption Changes and the Number of Bulk Pigments

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Electrochromic Absorption Changes, Photosynthetic Unit

A correlation between the amplitude of the electrochromic absorption changes measured at 480 nm and 520 nm and the size of the photosynthetic unit is found. Three tobacco mutants which differ with respect to their photosynthetic unit size exhibit decreased amplitudes of the electrochromic absorption changes which parallel the size of the photosynthetic units. Under these conditions the ATP-yield per flash is unaffected by the photosynthetic unit size. From this it is deduced that the correlation between the amplitude of the electrochromic absorption change and the photosynthetic unit size is due to the fact that the number of pigments undergoing an electrochromic bandshift is proportional to the total pigment content. As the influence on the electrochromic effect at 520 nm is too pronounced as to be simply explainable by the variation of the carotenoid content in the different mutants it is inferred that either structural effects leading to a modification of the carotenoid orientation in the mutants or an indirect influence of chlorophyll on the electrochromic bandshift which depend on the number of chlorophyll molecules are responsible.

Introduction

Among the various light induced absorption changes reflecting different types of molecular events of the photosynthetic apparatus those peaking at 480 nm (negative) and 520 nm (positive) are useful indicators of the electrical potential gradient across the thylakoid membrane (for review s. ref. 1–3). This indicator effect based on electrochromic bandshifts is widely used for the analysis of electrical phenomena in thylakoid and chromatophore membranes. These electrochromic absorption changes are kinetically labelled by their sensitivity to ionophores which enhance the membrane permeability selectively for specific ions. In this way the difference spectrum of the electrochromic effect was obtained for the whole visible range [4]. On the basis of this spectrum and by comparison with *in vitro* capacitor model systems [5] it was inferred that practically all types of bulk pigments (chlorophyll a and b, carotenoids) undergo electrochromic bandshifts. However, measurements on bacteria mutants led to the conclusion, that only a fraction of the total carotenoids contribute to the observed bandshift and that this pool is indistinguishable by spectroscopic methods [6]. As tobacco mutants are available, whose photosynthetic units, *i. e.*, the number of bulk pigments per reaction center, can be selectively modified in their size [7, 8], it seems to be worthwhile to investigate a possible correlation between the magnitude of the electrochromic effect and the bulk pigment content in chloroplasts of these plants.

The present measurements show, that the total amplitudes of the absorption changes at 480 nm and 520 nm, induced by single turnover flashes, correlate with the size of the photosynthetic units. The results indicate that the fraction of the bulk pigments contributing to the overall electrochromic effect is dependent on the total chlorophyll content. A possible explanation of this phenomenon will be given in the light of a recent model [16].

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Abbrev.: PSU, Photosynthetic Unit.

Materials and Methods

The isolation of photochemically active stroma-free swellable chloroplasts from *Nicotiana tabacum* var. John Williams Broadleaf (JWB) is described

in ref. 7, 9. The same procedure has been applied for the isolation of the chloroplasts from the tobacco mutants Su/su, Su/su var. Aurea (in previous papers referred to as Su/su[2]) and Consolation var. Aurea. The chloroplast preparations were used for the assay within 1 h after grinding the leaves.

The reaction mixture under phosphorylating conditions (referred to as coupling conditions) contained chloroplasts (100 μM chlorophyll for JWB; 33 μM chlorophyll for Su/su and 25 μM chlorophyll for Su/su var. Aurea and Consolation var. Aurea, respectively), 0.67 mM $K_3[Fe(CN)_6]$ as electron acceptor, 10 mM NaCl, 3.3 mM $MgCl_2$, 0.4 M sucrose, 1.67 mM ADP, 1.67 mM K_2HPO_4 and 10 mM Tricine-NaOH, pH 7.5. For measurements under basal conditions $MgCl_2$, K_2HPO_4 and ADP have been omitted. The average size of the photosynthetic units was obtained by polarographic measurements with a Clark-type electrode of the average oxygen yield per single turnover flash. The ATP-formation was determined by a counter technique applying ^{32}P-labelled substrate as described in ref. 10.

Excitation: white flashes (~ 20 μs duration) at a repetition rate of 1 Hz. In the ATP-experiments additionally a continuous background illumination of the same intensity and bandwidth as the detecting light beam for the absorption changes at 520 nm was applied. The absorption changes of the electrochromic effect were detected by a flash-spectrophotometer as is described in ref. 11. Excitation with short flashes (~ 20 μs) at a repetition rate of 1 Hz passed through a cut-off filter Schott RG10. 128 signals were averaged in a Fabri-Tek, model 1062. The electrical bandwidth ranged from 0 – 15 kHz.

The intensity of the measuring light beam was $100 - 300\ erg \cdot cm^{-2} \cdot s^{-1}$, optical bandwidth 5 nm, optical path length 1.3 mm. All experiments were performed at room temperature (20 °C).

Results and Discussion

For a correlation between the number of the bulk pigments and the amplitude of the electrochromic absorption changes the measured optical signals are normalized to the number of electron transport chains (rather than to the total chlorophyll content of the chloroplasts). In Table I the mean size of the photosynthetic units, measured as the average oxygen yield per single turnover flash and expressed in units of chlorophyll molecules per redox equivalent transferred through the electron transport chain, is compiled for different tobacco mutants. It is seen that Su/su var. Aurea and Consolation var. Aurea contain only about 25% of the chlorophyll per reaction center compared to the wild type JWB. These results are in close agreement with earlier measurements for the relative amplitude of absorption changes reflecting the number of system-II-reaction centers[12]. Additionally, the same effect is observed for the Hill-reaction rate (s. Table I). Hence, for the measurements of the absorption changes suspensions of chloroplasts from these mutants with a 4-fold lower total chlorophyll content have been used, in order to allow a direct comparison of the experimental data on the basis of the reaction center concentration. Likewise, the chlorophyll concentration of the Su/su chloroplast suspension was reduced to 33%.

Table I. Average size of the photosynthetic units, expressed as chlorophyll molecules per redox equivalent transferred through the electron transport chain, and Hill-reaction rate given in μmol O_2 evolved per mg chlorophyll and hour.

Tobacco variety	PSU $\left[\frac{\text{Chl-molecules}}{\text{redox eq.} \cdot \text{e-chain}}\right]$		Hill-rate $\left[\frac{\mu\text{mol } O_2}{\text{mg Chl} \cdot \text{h}}\right]$	
	Basal	Coupled	Basal	Coupled
John William's Broadleaf	300 ± 15	280 ± 20	150 ± 30	230 ± 30
Su/su var. Aurea	95 ± 15	70 ± 15	780 ± 50	1160 ± 30
Consolation	95 ± 15	85 ± 15	430 ± 20	730 ± 20

The electrochromic absorption changes detected at 520 nm are depicted in Fig. 1. It is seen that the amplitude drastically decreases in the chloroplasts of the mutants and nearly parallel the size of the photosynthetic units. Practically the same result is obtained at 480 nm (data not shown), but the effect at this wavelength appears to be even more pronounced. Thus, the data of Fig. 1 indicate that there exists as correlation between the size of the photosynthetic unit (PSU) and the amplitude of the electrochromic absorption changes. The overall electrochromic effect of an ensemble of pigments depends on 3 factors: a) the strength of the electrical field acting on the pigments, b) the orientation relative to the electrical field of the permanent electrical dipole moment resulting as the difference between the moments of the excited and the ground state, respectively, and c) the number and type of pigments undergoing an electrochromic bandshift. In spinach chloroplasts it was shown that the amplitude of the electrochromic absorption change

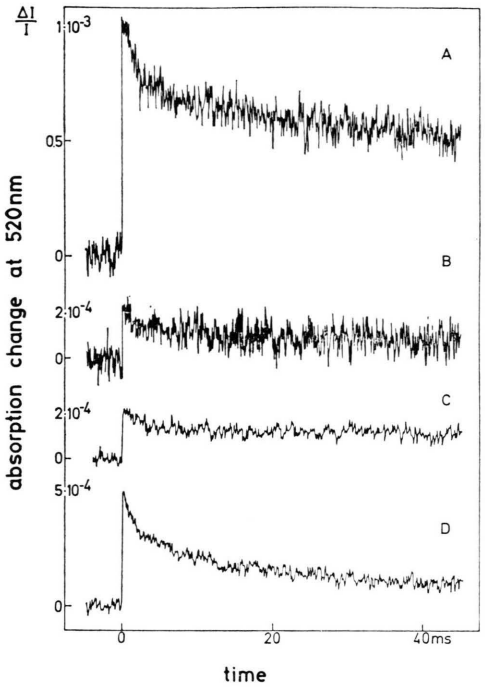

Fig. 1. Absorption changes at 520 nm in different tobacco chloroplasts. a) John William's Broadleaf, b) Su/su var. Aurea, c) Consolation and d) Su/su. All experiments were made under coupled conditions (s. Materials and Methods). The differences in the signal/noise ratio between experiments a,b and c,d, respectively, are caused by different optical density and turbidity of the samples.

at 520 nm linearly depends on the transmembrane electrical gradient $\Delta\varphi$ generated by vectorial electron transfer from the inside to the outside (for rev. s. ref. 1, 2). Hence, the first question, which remains to be answered, refers to the extent of the light induced electrical potential gradient $\Delta\varphi$ across the thylakoid membrane of wild type and mutant chloroplasts. Based on a simple model, $\Delta\varphi$ can be estimated [13] to:

$$\Delta\varphi = \frac{Q \cdot l}{S \cdot \varepsilon \cdot \varepsilon_0} ,$$

wherein Q = the number of electrons transferred per electron transport chain, S = membrane area per electron transport chain, l = the thickness of the insulating membrane layer and ε = the effective dielectric constant of this layer (phospholipid). In our case, Q ($=2$ for single turnover flashes) is constant for the different chloroplasts, because the electron transport chain remains intact, ε and l should be nearly invariant to the size of PSU, if a bilayer array of the same lipids is assumed to build up the insulating layer. Furthermore S should not increase, when the bulk pigment content decreases. Therefore, $\Delta\varphi$ per single turnover flash, symbolized by $\Delta\varphi^1$, is expected to be nearly invariant. In order to corroborate this rough estimation, the phosphorylation in single turnover flashes (time between the flashes 1 s) was measured, because under these excitation conditions a linear relationship between $\Delta\varphi^1$ and the average ATP-amount per flash was shown to exist [2]. The experimental data given in Table II clearly show that the ATP-yield per flash remains uneffected by the size of the photosynthetic unit. This favours the above mentioned consideration of $\Delta\varphi^1$ to be nearly the same in the different types of chloroplasts.

Table II. ATP-formation in isolated tobacco chloroplasts under repetitive flash excitation ($t_d = 1$s) and saturating continuous illumination (2 min white light 120 000 lx).

Tobacco variety	ATP/e-chain $\left[\frac{\text{ATP-molecules}}{\text{e-transport chain} \cdot \text{flash}}\right]$	ATP-rate $\left[\frac{\mu\text{mol ATP}}{\text{mg Chl} \cdot \text{h}}\right]$
John William's Broadleaf	0.15 ± 0.02	65 ± 10
Su/su	0.20 ± 0.04	335 ± 75
Su/ su var. Aurea	0.18 ± 0.06	455 ± 65
Consolation var. Aurea	0.16 ± 0.05	435 ± 55

Taking into account $\Delta\varphi^1 \approx$ const., the simplest explanation for the correlation between the amplitude of the electrochromic absorption changes and the PSU-size would be provided by the assumption that the number of pigments undergoing an electrochromic bandshift is proportional to the total pigment content. If one accepts that the electrochromic 520 nm absorption changes arise mainly due to carotenoid bandshifts, whereas at 480 nm chlorophyll b is predominantly responsible for this effect [14], then the more pronounced dependency on PSU-size of the 480 nm change could be rationalized by a variation of the concentration ratio of the different types of bulk pigments by the genetic mutation. Table III compiles the pigment content of the tobacco leaves [15]. It is seen that in comparison to the wild type JWB the relative carotenoid content normalized to the total chlorophyll amount is about 1.5 times higher in the mutant Su/su and 2 – 4 times higher in Su/su var. Aurea. On the contrary, the relative chlorophyll b concentration is smaller in the mutants. Taking into account the values for

Table III. Pigment content in leaves of the tobacco mutant *N. tabacum* Su/su var. Aurea.

Tobacco variety	Chlorophyll content per cm^2 leaf area [μg]				
	Chl_a	Chl_b	Total Chl	$\frac{Chl_a}{Chl_b}$	$\frac{\text{Total carotenoid}}{\text{Total chlorophyll}}$
young leaves					
John William's Broadleaf	17.34 ± 2.18	7.65 ± 0.67	24.99 ± 7.08	2.267	0.058 ± 0.001
Su/su	7.57 ± 0.49	2.59 ± 0.42	10.16 ± 0.9	2.915	0.103 ± 0.014
Su/su var. Aurea	2.49 ± 0.45	0.87 ± 0.43	3.35 ± 0.9	2.86	0.219 ± 0.013
fully expanded leaves					
John William's Broadleaf	32.6 ± 3.4	10.4 ± 0.6	45 ± 0.1	3.13	0.056 ± 0.001
Su/su	6.85 ± 1.75	1.16 ± 0.34	8 ± 2	5.9	0.079 ± 0.003
Su/su var. Aurea	4.35 ± 0.35	0.74 ± 0.16	5.08 ± 0.32	5.88	0.117 ± 0.006

the size of the photosynthetic units, given in Table I, these results show, that in young leaves the total number of carotenoids per electron transport chain is practically the same in the different tobacco varieties and decreases down to 50% in mature leaves of Su/u var. Aurea compared to JWB. A much more drastic effect is observed for the chlorophyll b content. It amounts to only about 25% in young Su/su var. Aurea leaves (in comparison to JWB) and falls down to 15% in fully expanded leaves. A corresponding decrease is also observed for the amplitude of the negative absorption change at 480 nm. This favours the assumption that the electrochromic effect at 480 nm is mainly a chlorophyll-b bandshift. On the other hand, the influence on the electrochromic effect at 520 nm is much too pronounced as to be simply explainable by the variation of the carotenoid content in the different mutants. Hence, one can infer that there exist either structural effects leading to a modification of the carotenoid orientation in the mutants or an indirect influence of chlorophyll on the electrochromic carotenoid bandshift, which depends on the number of chlorophyll molecules. The latter assumption would be in accordance with a recent model claiming the complexation of lutein with chlorophyll-b and chlorophyll-a to be responsible for the pseudo-linear electrochromic effect at 520 nm caused by carotenoid [16]. If one accepts that only carotenoids complexed with chlorophyll-b (and Chl-a) markedly contribute to the electrochromic 520 nm absorption change and that the degree of complexation is nearly proportional to the chlorophyll concentration, then the correlation between the 520 nm amplitude and the size of PSU becomes understandable, despite of the comparatively high carotenoid concentration. In order to try to clarify the action of the carotenoids, the effect of antibodies specifically reacting with lutein, neoxanthine, zeaxanthine and β-carotene, respectively, have been investigated. However, none of these antibodies exerted any effect on the 520 nm absorption change in chloroplasts of the wild type tobacco JWB. Hence, it is inferred, that binding of these specific proteins to the thylakoid membrane leading to a partial reduction of the electron transport rate [17, 18] does not seriously modify the structural arrangement as well as the optical properties of the carotenoids which undergo an electrochromic bandshift. Irrespective of the mechanistic details, the present results indicate, that there exists a strong correlation between the amplitude of the electrochromic absorption changes measured at 480 nm and 520 nm, respectively and the size of the PSU. However, the electronic response of the carotenoids appears to be governed by some special type of interaction with the bulk chlorophylls. In this respect, it is very interesting to note latest findings of Akoyunoglou [19] which provide some evidence for a change of the sign of the electrochromic carotenoid band-shift during the ontogenetic development of chloroplasts. Further experiments are required to clarify the interrelation between the structural organization of the bulk pigments and the electrochromic response of the carotenoids.

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