Regular paper

Electrochromic absorbance changes in the chlorophyll-c-containing alga *Pleurochloris meiringensis* (Xanthophyceae)

Claudia Büchel1 & Gyöző Garab2
1Institute for General Botany, University of Mainz, 55099 Mainz, Germany; 2Institute of Plant Biology, Biological Research Center, 6701 Szeged, Hungary

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**Abstract**

Flash-induced absorbance changes were measured in the Chl-c-containing alga *Pleurochloris meiringensis* (Xanthophyceae) between 430 and 570 nm. In addition to the bands originating from redox changes of cytochromes, three major positive and two negative transient bands were observed both 0.7 and 20 ms after the exciting flash. These transient bands peaking at 520, 480 and 451 nm and 497 and 465 nm, respectively, could be assigned to an almost homogeneous shift of the absorbance bands with maxima at 506, 473 and 444 nm, respectively. The shape of the absorbance transients elicited from PSI or PS II was identical, and the two photosystems contributed nearly equally to the absorbance changes. Furthermore, the decay transients were sensitive to the preillumination of the cells. These data strongly suggest that the absorbance transients originate from an electrochromic response of carotenoid molecules. The pigment species responsible for the 506 nm absorption band, probably heteroxanthin or diatoxanthin, transferred excitation energy to both photosystems as shown by the aid of 77 K fluorescence excitation spectra.

**Abbreviation:** LHC – light-harvesting complex

**Introduction**

Upon illumination of photosynthetic membranes positive and negative charges are separated in the reaction centre complexes. The primary charge separation is followed by a vectorial transport of charges which is accompanied by uptake and release of protons on opposite sides of the membrane vesicle. As a result, a transmembrane electrochemical potential builds up, which consists of a proton concentration gradient, ΔpH, and an electrical potential difference, ΔΨ. By a reverse flow of protons the electrochemically stored energy is consumed in ATP synthesis (Mitchell 1974).

The rise and decay of the transmembrane electrical potential difference can be followed by observing the electrochromic absorbance change of the pigments embedded in the membrane. The pigments exposed to the electric field undergo an almost homogeneous absorption band shift, the so-called electrochromic or field-indicating shift which is proportional to the field strength (for review see Junge 1977; Witt 1979). Electrochromic absorbance changes induced by single turnover flashes provide useful information on the concentration of active reaction centres (e.g. Junge and Jackson 1982), on the secondary charge transport, e.g. in the Cyt b6f complex (Cramer et al. 1987), and on various factors affecting the permeability of membranes e.g. activation of ATPase (Girault and Galmiche 1976; Morita et al. 1983; Schreiber and Rienits 1982).

The detector molecules with and without permanent dipole moment respond linearly or quadratically, respectively, to a homogeneous transmembrane electric field. Local or pre-existing fields can significantly alter the sensitivity of some pigments. For example, polar chlorophyll molecules close to
Carotenoids induce dipole moments in the latter which change the quadratic response to a pseudo linear field-dependence. The sensitivity of the absorbance bands of pigments also depends on the orientation of their transition dipoles with respect to the electric field vector (Paillotin and Breton 1977). Hence, different pigments do not respond equally to the electric field and in fact usually only a relatively small fraction of molecules, the 'field-indicating pigments', exhibit large, well discernible electrochromic response (Joliot and Joliot 1989). Moreover, in some membranes, e.g. in heterocysts (Houchins and Hind 1983) and unicellular cyanobacteria no flash-induced electrochromic absorbance change has been identified between 450 and 540 nm where the electrochromic bands can most easily be observed in green algae and higher plants.

In higher plants and green algae, the electrochromic shift exhibits major negative and positive maxima at 478 nm and 515 nm, respectively (Duysens 1954; Witt 1955). Schmidt et al. (1971) showed that Chl-b is responsible for the major negative band at 478 nm, while the transient absorbance band around 520 nm could be correlated with the interaction of Chl-b and lutein in the complexes (Sewe and Reich 1977).

\textit{Pleurochloris meiringensis}, an alga belonging to the Xanthophyceae, contains neither Chl-b nor lutein. Instead of Chl-b a small amount of Chl-c is present as accessory pigment in the light-harvesting complexes. The main carotenoid diadinoxanthin is accompanied by heteroxanthin, vaucheriaxanthin-ester and diatoxanthin (Büchsel and Wilhelm 1993). Thus, the electrochromic absorbance changes whose existence has not been shown in this organism are expected to be different from those in green algae and higher plants.

In this work, by using single turnover flashes, we have identified the main absorbance transients between 450 and 540 nm in \textit{P. meiringensis}. We show that the absorbance changes can be fitted with the assumption of a homogeneous shift of the absorbance bands with maxima at 506 nm, 473 nm and 444 nm, respectively. The nearly equal contribution of the two photosystems to the absorbance changes, and the sensitivity of the decay kinetics to preillumination strongly suggest that the transients originate mainly from an electrochromic shift of carotenoid molecules. The band at 506 nm could also be identified in the excitation spectrum of low temperature fluorescence emission, and the carotenoid species could be located in both LHC I and LHC II.

\textbf{Materials and methods}

\textit{Pleurochloris meiringensis} Vischer (Culture collection Göttingen, n° 860-3) was grown as batch culture in a nutrient medium according to Böger (1969) in white light of 15 \(\mu\)E m\(^{-2}\) s\(^{-1}\) intensity. Cells were harvested in the logarithmic growth phase and concentrated by sedimentation. Chlorophyll content was measured after homogenisation of the cells in acetone (90%) according to Jeffrey and Humphrey (1975).

For absorption transient measurements the cells were used in nutrient solution supplemented with 0.02 M HCO\(_3\)\(^{-}\); 5% Ficoll was added to avoid sedimentation of the cells during measurement. The Chl-a concentration of the samples was adjusted to 70 \(\mu\)g/ml. Unless stated otherwise, the cells were dark-adapted prior to measurements for at least 20 min. All measurements were performed at room temperature.

Absorption transients were induced by saturating flashes of red light (3 \(\mu\)s duration at half peak emission) in a set-up described earlier (Barabás et al. 1985). Data were collected in a digital storage oscilloscope (TEK 2224, Tektronix) and processed in a personal computer. The time resolution of the instrument was adjusted to 100 \(\mu\)s. Flashes were given at a frequency of 1 s\(^{-1}\) and 30 kinetic traces were averaged.

For spectral analysis, kinetic measurements were carried out at different wavelengths between 430 nm and 570 nm and analysed 700 \(\mu\)s after the exciting flashes. At 700 \(\mu\)s, between 450 and 540 nm the contributions of the absorbance changes due to redox reactions of Cyt b\(_{6f}\) (Wasserman 1980) and P700 (Hiyama and Ke 1971) were small compared to the measured amplitudes. In this region the transient spectra were deconvoluted by mathematical fitting with the assumption that the transients are predominantly of electrochromic nature and thus can be fitted with a linear combination of first derivatives of gaussian absorbance bands which undergo bathochromic shift:

\[\frac{dA}{d\nu} = -(k \cdot (\nu - \nu_0))/\sigma^3 \cdot \sqrt{2\pi} \cdot e^{-(\nu - \nu_0)^2/2\sigma^2}\]