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Wavelength dependence and kinetics of the photovoltaic effects in chloroplast suspensions

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Abstract

The photovoltaic effect in chloroplast suspension is studied using laser pulses of various wavelengths in a single experimental setup. The polarity of the photovoltage depends on the wavelength of the laser pulse rather than the differences between laser and flash-lamp pulses. At the long-wavelength side of the visible spectrum and between the two absorption bands of the chlorophyll the observed polarity is opposite to that expected from simple shading. Also, the decay kinetics of the signals are wavelength dependent. In some wavelength regions the signal has a positive, as well as a negative component. We show that swollen chloroplasts exhibit understandably different photovoltaic effects. This observation suggests, that polarity depends on the geometry of the chloroplasts and support the theory, that the interference pattern of the light distribution determines the polarity of the signal.

Keywords: Photovoltaic effect; Polarity; Chlorophyll

1. Introduction

The transient photovoltage which can be detected after a non-saturating light flash through a pair of electrodes immersed into a chloroplast suspension, is supposed to be caused by the primary charge separation. According to the "light gradient" interpretation [1–3] the sides of thylakoid vesicles that face the light source absorb more light than those shaded by them. Thus, at non-saturating conditions the charge displacements that occur in the two halves of the vesicles do not compensate each other [1–3].

This explanation of the photovoltage effect predicts a definite sign for the signal: the electrode farther from the light source has to become positive. (In the following we refer this polarity as positive.) This prediction is based on the well-established fact that during charge separation in the reaction center an electron is transferred from the inside to the outside of the thylakoid vesicle [4]. Indeed, the polarity was found positive in the early experiments using flash lamps as the light source [1–3].

Surprisingly enough an action spectrum of this signal published by Gräber and Trissl [5] showed a wavelength-dependence of the polarity. (These experiments employed a flash lamp and interference filters.) The polarity was positive in the blue and red absorption bands of chlorophyll and negative between them and at wavelengths longer than 700 nm. In swollen chloroplasts only the positive signal could be detected.

Later Trissl et al. [6] published a negative

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polarity signal generated by rubin and Nd–YAG lasers. According to this article the polarity of the signals generated by laser and by flash lamp differed even at the same wavelength. It was supposed, that the different duration of the two types of light flashes caused the difference. The laser-generated signal was so fast that any explanation other than that the signal originates from the primary charge separation, was excluded. (Signals from both photochemical reaction centers have a component with less than 50 ps rise time according to later articles of Trissl et al. [7,8].) The flash-lamp-generated signal was suspected to come from a different effect as it had been suggested earlier by Becker et al. [9]. Neither the "wrong" polarity of the supposedly physiological signal nor the dependence of the sign on the duration of flash were explained.

In our own experiment a laser-generated positive photovoltage was found at 420 nm excitation (Meszéna et al. [10]) but the negative polarity was confirmed using rubin and Nd–YAG lasers (Meszéna and DeVault [11]). All these measurements were made using the same measuring cell, preamplifier and preparation method.

This suggested that the same wavelength-dependent signal could be generated by both flash lamp and by laser flash, and thus that the wave-

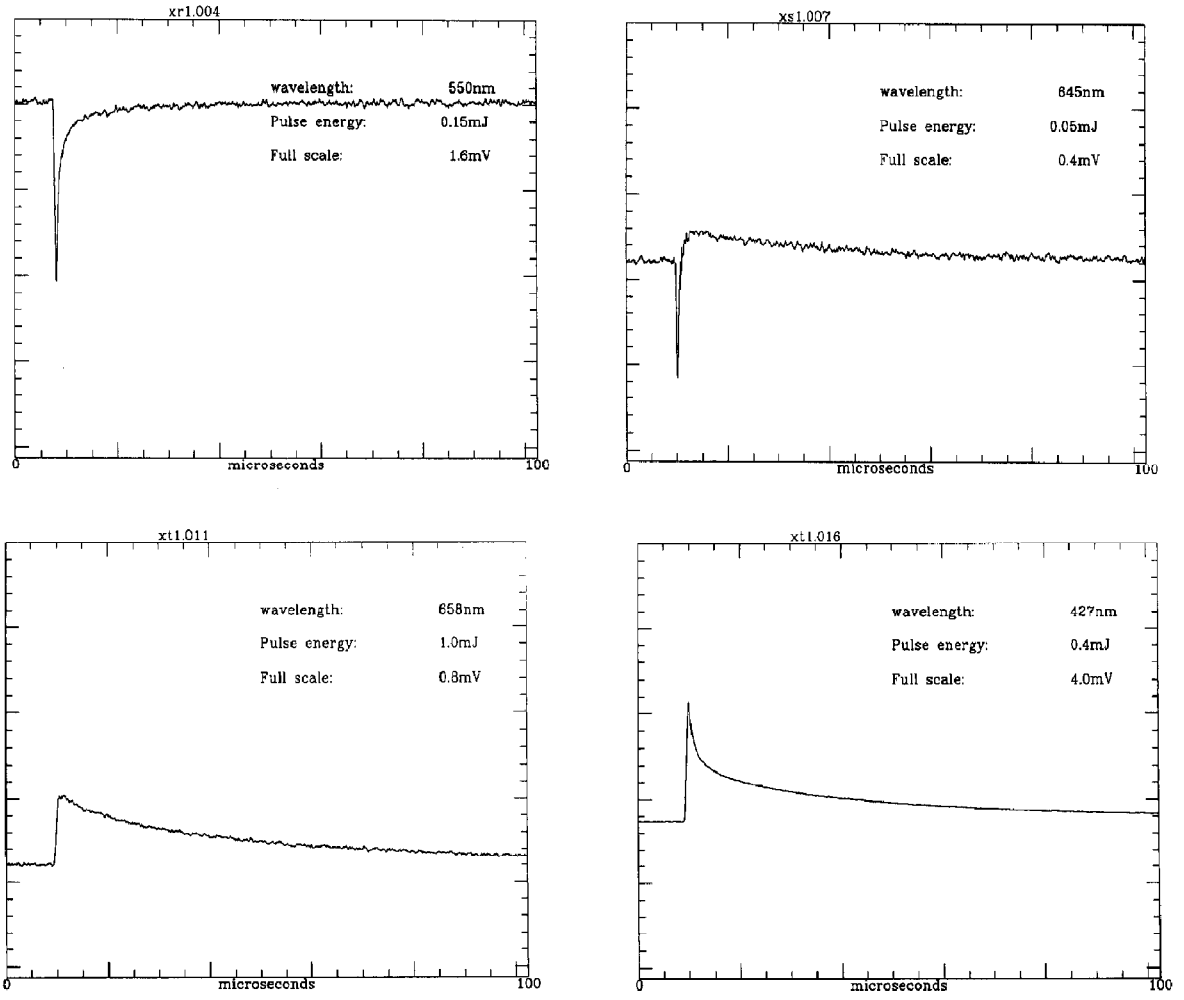

Fig. 1. Photovoltage signals from chloroplasts in 0.3 M sucrose solution. The kinetic traces were obtained by averaging 32 transients.

length rather than the flash duration may be the factor determining the polarity of the signal. In this work I confirm this suggestion by checking wavelength-dependence of the laser-generated signal using the same setup to generate positive and negative signals.

It was pointed out by Meszéna and DeVault [11], that using geometrical optics in this context is not justified, because dimensions of the thylakoid system are smaller than the wavelength. According to this concept, both the positive and the negative photovoltage signals are caused by the primary charge separation, and wavelength dependence of the light distribution caused by optical interference is responsible for the wavelength-dependent polarity. In order to check this prediction the interference effects were altered by carrying out experiments with swollen chloroplasts.

A quantitative analysis of the light distribution in the thylakoids is presented in the accompanying article [12].

2. Materials and methods

Chloroplasts were prepared from fresh spinach as described [10]. To keep the conductivity of the sample as low as possible chloroplasts were resuspended into a salt-free solution containing only 0.3 M sucrose. Chloroplasts were swollen by resuspension in distilled water. The suspension was stored on ice for less than three hours until the measurements. The measurements were carried out at room temperature. The chlorophyll concentration of the samples was about 1 μM.

The light source was a tunable dye laser pumped by an excimer laser with flash length around 20 ns. The following dyes were used: stilben 3 for the 425 ± 2 nm wavelength, fluorescein 27 for 536–610 nm, sulphorhodamin B for 610–645 nm, and DCM for 658 ± 2 nm.

The output energy was between 0.1–1.3 mJ per pulse depending on the dye and wavelength. Neutral density filters were used to attenuate the light. The illuminated area was about 1 cm^2 in the cuvette. The measuring cell and the preamplifier were the same as before [11]. The signals were recorded by a transient recorder and averaged by an IBM AT compatible computer. The time resolution was limited by the pre-amplifier to about 0.3 μs. The duration of the light pulse was around 20 ns.

To prove the physiological origin of the signal, heat-inactivated (70°C for 10 min) chloroplasts were used as a control. All of the possible artefacts could be excluded by checking the lack of the signal in the heat-inactivated sample.

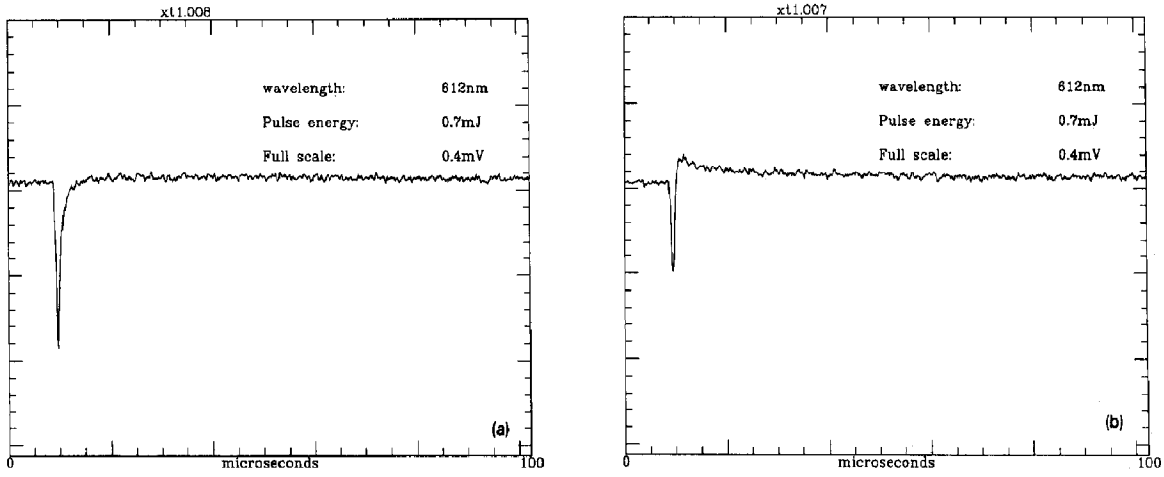

Fig. 2. Photovoltage signals from (a) control (in 0.3 M succrose) and (b) swollen chloroplasts at the same wavelength of the exciting laser flashes. Average of 32 transients.

3. Results and discussion

Fig. 1 shows the photovoltaic effect obtained with a suspension of chloroplasts in 0.3 M sucrose. The signal polarity and kinetics are strongly wavelength dependent.

Positive signals were detected around 425 and 658 nm, that is, in the two absorption peaks of the chlorophylls. The decay of these signals had a slow component of ≈ 50 μs. There is a kinetic difference between the two peaks: the signal detected around 425 nm has also a fast component, while the signal at 658 nm does not.

Negative signals were found with fast decay between 536 and 645 nm. The negative signal decayed with a half-time between 1 and 5 μs. In the range 610–645 nm the slow positive component was also detected, so the signal was bipolar in this region.

Fig. 2 shows the comparison between control (in 0.3 M sucrose) and swollen chloroplasts at 612 nm. Swelling of the chloroplasts changes the photovoltaic effect qualitatively. At this wavelength the signal from the control chloroplasts is negative, but that from the swollen chloroplasts is bipolar.

These signals were detected in the same setup, therewith excluding electric artefacts. The duration of the light flashes was not measured at every wavelength, but because a single laser was used, it should not change significantly. Fig. 3 shows a comparison between polarity data obtained here and in the literature. The laser polarity data measured here (including the presence of the bipolar signal) fit well to the flash lamp action spectrum obtained in ref. [5]. All of the data are in accordance with the hypothesis that for a given chloroplast preparation the wavelength of the exciting light determines the polarity independently from the duration of the exciting flash.

This conclusion is in contradiction with the result of Trissl et al. [6] in which laser excitation generated a signal with a polarity opposite to that generated by a flash lamp even at the same wavelength. This statement was based on the observation of a negative signal generated by a 694 nm ruby laser flash and a positive signal generated by a 689 ± 7 nm IF-filtered flash lamp.

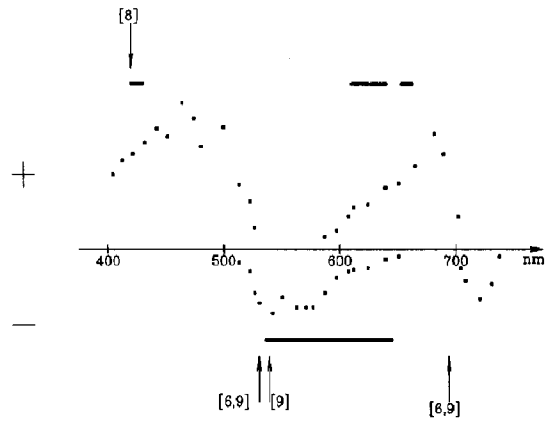

Fig. 3. Photovoltage polarity as a function of wavelength: comparison of various experimental results. Every item above/below the wavelength scale represent a positive/negative polarity. Discrete points represent the action spectrum measured by flash lamp [5]. The vertical arrows with serial numbers of references indicate wavelengths where positive or negative laser-generated signals were detected previously. Horizontal bars indicate polarity of signals observed in the present work.

But the action spectrum of Gräber and Trissl [5] exhibits a sharp polarity change just in this region, so the 14 nm bandwidth of the interference filter may have been too wide to establish the mentioned conclusion.

In the early experiments with polychromatic flashes probably the poor time resolution caused by the long duration of the light flash made it impossible to detect the very short negative component.

The picosecond rise time of the negative signals at 530 nm and 694 nm proves, that they must be directly connected to the primary charge separation [6–8]. The possibility, that the positive signal comes from the different mobilities of the positive and negative charges (as was proposed by Becker et al. [9]) can be excluded. The at least two orders of magnitude difference between the time constants of rise and decay is too large for this explanation (Meszéna et al. [10]). Otherwise, the positive polarity is more readily explained by primary charge separation, than the negative one, so the hypothesis, that the positive signal also comes from that source, is very attractive.

The fact, that swollen chloroplasts with modified geometry show a different signal (cf. figs. 2a

and 2b) is in line of the hypothesis [11], that an interference pattern determines the polarity. But interference effects alone cannot explain the observed differences in the kinetics of the signals. (It is worthwhile to note, that if the two kinetics were the same, the bipolar signal could never be seen.)

We suggest the following picture to interpret the negative polarity. At some wavelengths light intensity at the sheet nearer to the light source may be decreased by destructive interference between the incident light and the light reflected from the far sheet. If this effect is strong enough, an opposite light intensity difference, and consequently, an opposite polarity signal is expected. We expect normal, i.e. positive polarity within the main absorption bands, where the shadow of the front sheet (making the light intensity difference proposed by the light gradient theory) is the darkest.

A more detailed analysis shows, that an opposite polarity signal is expected from the stroma-membrane region at the long-wavelength side of the absorption bands [12]. The fact that the PSII contributes to the negative signal only at destacking conditions [6] also suggests that the negative signal comes from the stroma-membrane.

To interpret the kinetic differences between the positive and the negative signals we have to suppose, that the ion relaxation in the grana stack is slower than in the stroma-thylakoid. This hypothesis seems to be reasonable because of the presence of the appressed membrane regions in the grana stack.

A more detailed explanation of the experimental facts requires quantitative modelling of ionic relaxation kinetics.

After submitting this article we were informed about the paper of Paillotin et al. [13]. In this work wavelength-dependent polarity of the photovoltage signal is demonstrated by picosecond flash excitation. Detection of positive signal with that time resolution supports our conclusion, that it comes from the primary charge separation, too. They measured the PSI signal at stacking condition. Getting a positive signal in such an experiment does not exclude our suggestion, that it is generated mainly in the grana stack. PSI particles in the non-stacked top and bottom layer of the grana stack can generate considerably large (positive) photovoltage because of the large amount of absorbing material between them.

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