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Flash-Induced Absorption Change at 703 nm in Intact Leaves of Kidney Bean Plants*

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Abstract : The absorption change at 703 nm induced by flash actinic light in intact leaves of kidney bean plants was measured. The dependency of the absorption change upon the intensity of the detecting light and intensity and wavelength of actinic flash was examined in order to investigate photosystems and electron transport system *in vivo*. The light saturation of P700 oxidation and reduction by flash light was represented by hyperbolic curves, while that of P700 oxidation by detecting light was represented by an exponential curve. The action spectra of P700 oxidation and reduction at shorter wavelengths than 600 nm fluctuated in almost the same pattern, showing a minimum at 481 nm and a peak around 450 nm. Apparent differences between the two were observed above 630 nm. The action spectrum of P700 oxidation showed a peak at 621 nm, decreased at 683 nm and showed another peak at 700 nm, while the spectrum of P700 reduction decreased above a wavelength of 660 nm. Based on the analysis by the present method, the photoreactions observed in leaves with dense pigments were discussed.
Key words : Absorption change, Flash photolysis, Intact leaf, Kidney bean, P700.

インゲンマメの生葉において閃光によって誘起される 703 nm の吸光度変化について：狩野広美・小泉美香・桂　直樹*・稲田勝美**（農業生物資源研究所・* 現野菜茶業試験場・** 現鳥取大学砂丘利用研究施設）
要　旨：生葉に閃光を照射すると，P700 の酸化・還元による吸光度変化（700 nm 付近）が観察される．P700 の吸光度変化に対する測定光（703 nm）強度の影響および，閃光の強度と波長の影響を検討し，インゲンマメの生葉中における光化学反応と電子伝達の機構を解析した．閃光照射による P700 の酸化・還元反応の光飽和曲線は双曲線となったが，測定光強度と P700 の酸化量の関係は指数関数となった．閃光による P700 の酸化と還元の作用スペクトルは，600 nm 以下の波長域ではほとんど同じであり，481 nm に最小値を，そして 450 nm 付近にピークを示す．しかし，630 nm 以上の波長域では明らかに異なっている．すなわち，P700 の酸化の作用スペクトルは，621 nm にピークを示し 683 nm で一度低下するが，700 nm にもう一つのピークを示した．一方，P700 の還元の作用スペクトルは，660 nm 以上の波長域で低下した．
なお，フラッシュ・フォトリシス法による P700 の吸光度変化の解析により，濃厚な光合成色素を持つ作物の生葉中で起こる光化学反応について考察した．
キーワード：インゲンマメ，吸光度変化，生葉，フラッシュ・フォトリシス，P700.

The reaction center of photosystem I, P700 was found to exhibit bleaching upon illumination at a wavelength near 700 nm[11,13,23] for algae and plants. Many findings about photoreaction and photosynthetic electron transport were obtained by analysing the absorption change associated with P700 oxidation and reduction. As for higher plants, the characteristics of the absorption change were examined extensively using isolated chloroplasts and subchloroplast particles[14]. On the other hand, there are only a few reports on such a change[2,5,18] in the case of the oxidation and reduction of P700 in intact leaves, where the structures surrounding chloroplasts are tightly organized and markedly restrict the reaction of P700 which varies with the conditions in the cells. Moreover, the arrangements of cells in leaf tissues and of organelles in individual cells are also expected to affect photoreactions by controlling light conditions around the chloroplasts.

In the present investigation, the absorption change at 703 nm in intact leaves was examined in order to know the reactions of photosystems and electron transport system *in vivo* in more detail using flash photolysis in the

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hope that the result was to be a help in controlling light environments in agricultural crops. The absorption change at 515 nm, i.e. the electrochromic shift, which was reported by Morita et al.[16] in intact leaves was also examined in addition to the absorption change at 703 nm. The limitations in the measurements in intact leaves and the effects of leaf structures on the photoreactions are discussed.

Materials and Methods

Plant materials

Kidney bean plants (*Phaseolus vulgaris*) were grown in a growth chamber with artificial light at 25°C in the day time and 20°C at night. Other plants were grown in growth chambers under controlled conditions suitable for each plant.

Measurements of flash-induced absorption changes

Absorption changes induced in intact leaves were measured with a flash spectrophotometer (Union Giken Kogyo Co. Ltd.) which was described in detail in another report[10]. A leaf piece (1×2 cm) held between two plastic plates was fixed at 45° angle on an aluminum block and exposed to both detecting light and actinic flash light. Strong flashes (80 μs duration, maximum 50 J•m^{-2} per flash) were provided by a Xe lamp (Sugawara SF-U50) through cut filters, Mazda VB2B (400 nm—600 nm) and Toshiba R-60 ($\lambda > 600$ nm), and various interference filters (401 nm—721 nm, Nihon Shinkuu Kogaku Co. Ltd.). The interval of the serial flash lights was 20 s. Signals of 16 or 32 times were averaged to improve the signal-to-noise ratio.

For the measurements of action spectra, the energy of actinic flash lights of various wavelengths was measured by an energy meter (LI-COR LI-188B) and lowered and made even using a combination of neutral density filters. The relative intensity of the flash light was also decreased by using neutral density filters in the experiments of the light saturation curve. All the measurements were carried out at 25°C. Amplitude of absorption changes was indicated by the change in transmittance ($\Delta I/I$).

Chlorophyll contents were measured according to the method of Arnon[1].

Results

Absorption change induced by actinic flash light

Spectral absorption changes induced in an intact leaf were measured at 0.5, 2.0 and 60 ms after flashing (Fig. 1). Three obvious absorption changes were detected at around 515, 683 and 700 nm. No clear absorption change was detected at wavelengths shorter than 500 nm, where the bleaching due to P700 is anticipated[7]. The absorption increase around 515 nm corresponded to the electrochromic shift which was also reported with intact maize leaf[16]. The apparent absorption change at 683 nm which decayed within 2.0 ms was considered to correspond to the fluorescence of chlorophyll[3]. The absorption change at around 700 nm was that of P700[13,19,20], the maximum of which has been observed at 703 nm in most cases.

Typical traces of signal monitored at 515 and 703 nm against time before and after flashing are shown in Fig. 2, which are corresponding to the absorption changes at 515 and 703 nm in Fig. 1. The shape of the absorption change at 703 nm (top) was complicated while that at 515 nm (bottom) looked like a simple free decay signal. The transmittance at 703 nm which increased immediately after flashing, subsequently decreased beyond the level before flashing and then increased again slowly (top). Rumberg[19,20] suggested that the fluctuation of the signal indicated that P700 was partly oxidized due to the detection before irradiation by actinic flash light, because the detecting light acted as an actinic light and was subsequently reduced by the electrons from photosystem II and re-oxidized by the continuous detecting light after the interruption of the inflow of electrons.

Taking into account the movement of the signal, the base line (BL) of the absorption change was assumed to be that illustrated in Fig. 2, and signal amplitudes at 700 nm and 703 nm were logarithmically plotted against time (Fig. 3). The reduction of P700 appeared as a first order reaction. The half-life times of its decay in several plant leaves containing various amounts of chlorophyll which ranged from 7.0 ms to 11.2 ms are listed in Table 1.

The absorption changes at 515 nm disappeared almost at the same time as the end of P700 reduction (Fig. 2). However, the

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relaxation of high energy state was reported not to have a direct relation to the electron transport[16]. Both absorption changes at 515 nm and 703 nm were no longer observed in the presence of DCMU (3-(3′, 4′-dichlorophenyl)-1, 1-dimethylurea) at a concentration of 10^{-5} M, which blocks photosynthesis completely. Strong detecting light used in the current investigation was considered to oxidize all P700 resulting in the absence of further oxidation of P700.

Dependency of the absorption change at 703 nm upon the intensity of detecting light

The effects of the intensity of the detecting light at 703 nm on P700 oxidation (Pox in Fig. 2, top) were examined. The amount of P700 oxidized by the actinic flash light represents the amount of P700 which is not oxidized by the detecting light. The value increased with the decrease of the intensity of the detecting light (Fig. 4), as reported for spinach chloroplasts[19], intact chlorella[20] and blue green algae[13]. Fig. 5 shows the logarithmic plot of the amount of P700 oxidized by the actinic flash light against the intensity of the detecting light. All the points fell on a straight line. The intercept (Ptotal) corresponds to the total P700 contained in the leaf. The amplitude of the absorption change representing the total content of P700 is indicated in Fig. 4 by the arrows of Ptotal. This finding shows that the electrons from photosystem II are able to reduce all of P700.

The amplitude of the absorption change of total P700 in several plant leaves was measured by the above-mentioned method. Amounts of P700 calculated assuming that the extinction coefficient is 64/mM[7] were 75.0 to 105.3 pmol per unit leaf area (cm^2) and the chlorophyll/P700 ratios ranged from 418 to 528 (Table 2).

Dependency of absorption changes at 515 nm and 703 nm upon the wavelength of actinic light

The action spectra of P700 oxidation and reduction are shown in Fig. 6. The signals of P700 oxidation (Pox) and reduction (Pred) by flash light are illustrated in Fig. 2 (top). The action spectrum of P700 oxidation showed a peak at 452 nm and was minimum at 481 nm. It rose at longer wavelengths, reached a peak around 620 nm, decreased towards 683 nm and showed another peak at 700 nm. The action spectrum of P700 reduction was similar to that of P700 oxidation at wavelengths shorter than 600 nm. It reached a maximum at 636 nm and abruptly declined above 660 nm. According to Rumberg[19,20], the amplitude of P700 oxidation indicates the activity of photosystem I and that of P700 reduction corresponds to the activity of photosystem II. Although the possibility of the participation of cyclic electron transport associated with cyclic photophosphorylation was not excluded, P700 is surmised to be reduced by the electrons from photosystem II based on the fact that kinetics of electron transport was composed of one phase (Fig. 3). Therefore, action spectrum of P700 oxidation was considered to indicate that of photosystem I and P700 reduction, photosystem II, respectively.

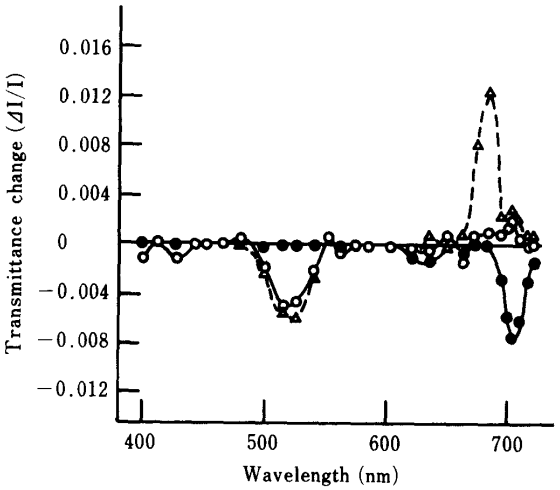


Fig. 1. Flash induced spectral changes in a kidney bean leaf.
Xe flashes through a VB2B filter (400 nm—600 nm) and a R-60 filter ($\lambda >$ 600 nm) were used for the measurements from 607 nm to 721 nm and from 401 nm to 582 nm, respectively. Absorption changes were measured at 0.5 ms (··· △ ···), 2.0 ms (— ○ —) and 60 ms (— ● —) after flashing.

The action spectrum of the absorption change at 515 nm (E in Fig. 2, bottom), was rather flat in the range of 540 to 700 nm but steeply decreased in the outside of this range (Fig. 7). The action spectrum was minimum at 481 nm and exhibited another hump between 411 and 466 nm. The absorption change at 515 nm, electrochromic shift, has close relation with the energy conservation in the thylakoid membrane by photoreactions and the operation of either photosystem I or

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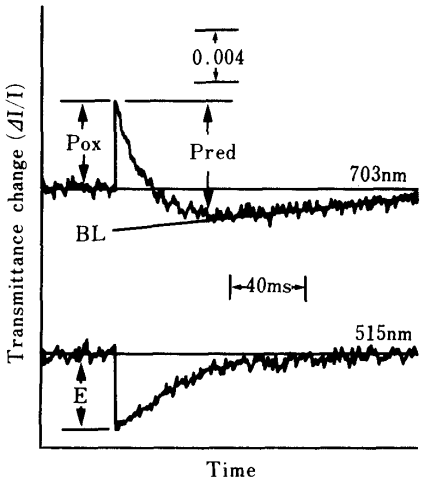


Fig. 2. Flash induced absorption changes at 703 nm (top) and at 515 nm (bottom) in a kidney bean leaf.

Pox, the amplitude of P700 oxidation by flash light; Pred, the amplitude of P700 reduction by the electrons from photosystem II; BL, the base line of absorption change for half-life time; and E, the amplitude of the electrochromic shift.

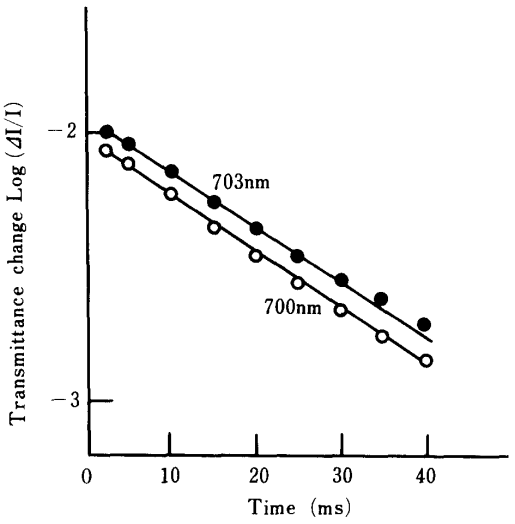


Fig. 3. Semilogarithmic plots of absorption changes at 700 nm and 703 nm against time in a kidney bean leaf.

The amplitudes of absorption changes were measured assuming that BL shown in Fig. 2 is the base line.

Table 1. Half-life times of P700 reduction in plant leaves containing various amounts of chlorophyll.

Plant		Chlorophyll content nmol·cm^{-2}	Half-life time ms
Kidney bean	(Young)	40.6	11.0
	(Fully developed)	46.9	8.0
	(Old)	30.2	8.2
Broad bean	(Very young)	37.1	7.1
	(Young)	41.6	7.4
	(Fully developed)	60.2	8.4
Pea	(Fully developed)	46.3	9.7
Turnip	(Very young)	26.3	8.7
Spinach	(Fully developed)	51.9	7.0
Soybean	(Very young)	37.6	11.2

photosystem II is needed for the induction of it[22].

Dependency of the absorption change at 703 nm upon the intensity of actinic light

Effects of the intensity of flash light on P700 oxidation (Pox) and reduction (Pred) were examined using leaves containing 30 and 51 nmol chlorophyll per cm^2 leaf area (Fig. 8). The amplitudes of P700 oxidation and reduction increased with the increase of the intensity of the flash light, and they were saturated. The relationship between the flash intensity and the extent of P700 oxidation or reduction could be expressed by hyperbolic curves judging from the fact that their Hofstee plots were straight lines (Fig. 9).

Discussion

Absorption changes due to P700 oxidation and reduction and electrochromic shift in

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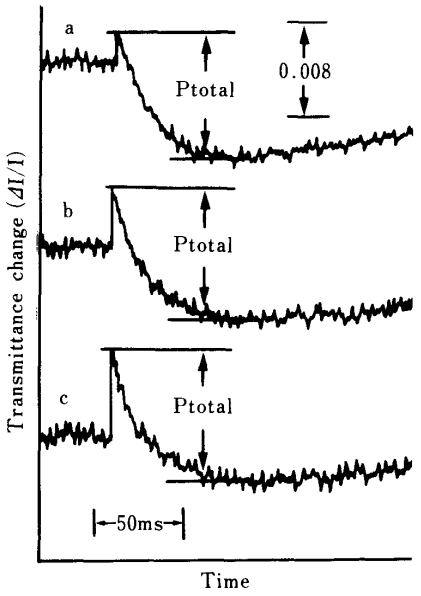


Fig. 4. Dependency of the absorption change at 703 nm in a kidney bean leaf upon the intensity of detecting light.

Relative intensities of the detecting light were a, 100% (0.20 W•m^{-2}) ; b, 39.0% and c, 20.5%. The amplitudes of the absorption change corresponding to the total amount of P700 are indicated with the arrow of Ptotal.

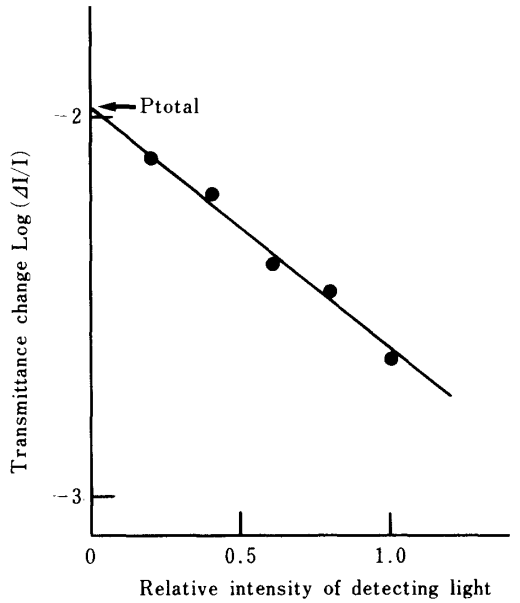


Fig. 5. Semilogarithmic plot of the amount of oxidized P700 by actinic flash (Pox) against the relative intensity of detecting light.

The results of Fig. 4 were plotted. Intensity of the detecting light of 1.0 was 0.2 W•m^{-2}.

Table 2. P700 contents and chlorophyll/P700 ratios in intact leaves.

Plant	P700 content pmol•cm^{-2}	Chlorophyll content nmol•cm^{-2}	Chlorophyll/P700
Kidney bean	75.0	33.4	445
	82.5	35.1	425
	78.7	32.9	418
Soybean	86.0	37.8	439
Turnip	75.0	39.6	528
Spinach	105.3	49.4	469

intact leaves were examined by using a flash spectrophotometer.

Haehnel et al.[6] stated that there were three phases in P700 dark reduction with different half-life times, 10 μs, 200 μs and 20 ms, respectively. The former two phases ascribed to the electron inflow from plastocyanin and cytochrome *f* were too short to be measured by the apparatus used in the current investigation, because that the use of a strong flash of 80 μs duration to saturate the photoreactions in intact leaves made the signal within 100 μs being out of scale. The signal which was analyzed corresponded to the last phase ascribed to the electron transport from photosystem II through the rate limiting step of plastoquinone. Accordingly, as a strong detecting light was employed, the dark reduction of P700 showed only one slow phase (Fig. 2) by maintaining plastocyanin and cytochrome *f* in an oxidized state[6]. This finding is supported by the facts that the half-life times of P700 reduction (from 7 ms to 11 ms) (Fig. 3, Table 1) corresponded to those of the electron inflow from plastoquinone in isolated chloroplasts and that the amount of P700

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reduced during the above-mentioned slow phase coincided with the total amount of P700 contained in the leaf (Fig. 4).

The effect of the intensity of actinic flash on P700 oxidation and reduction (Fig. 8) did not correspond to that of the detecting light (Fig. 4). The light saturation curve of the former was hyperbolic (Fig. 9) while that of the latter was exponential (Fig. 5). The discrepancy can be ascribed to the differences in the conditions of the two experiments. In the former the flash light which was used after a long interval acted on the completely relaxed photosystem I and II as a strong excitation independently. On the other hand, in the latter case, the detecting light acted as a continuous excitation in a steady state, where the sequentially arranged two photosystems were not relaxed. As suggested by the effects of DCMU, these systems did not react independently to the continuous light.

A similar tendency in the light saturation curves was reported by several authors. Kok[12] reported that in intact chlorella O_2 evolution by flash light with a short interval or by continuous light responded exponentially to the intensity of light. On the other hand, Rieske et al.[17] and Satoh et al.[21] using chloroplasts reported that there was a hyperbolic relationship between the rate of Hill reaction and the intensity of actinic light. In these cases, the excessive amount of electron acceptor added broke the sequential arrangement between the two photosystems.

The measured action spectra of photosystem I and photosystem II did not fully correspond to the absorption spectra of pigments likely to be involved (Fig. 6). In the current investigation, as intact leaf segments containing dense pigments (approximately 30 nmol chlorophyll per cm^2 leaf area) were used as materials the action spectra did not depended on the absorption efficiencies of photosynthetic pigments. Therefore, the contribution of photosynthetic pigments to each of the two systems could not be clearly determined, but the effectiveness of monochromatic lights on the photosystems in living leaves was indicated. However, if it is considered that about 60% of P700 was oxidized by the detecting light before excitation, the distribution of light energy is 2 or 3 times more favorable for photosystem II in this experiment, as observed

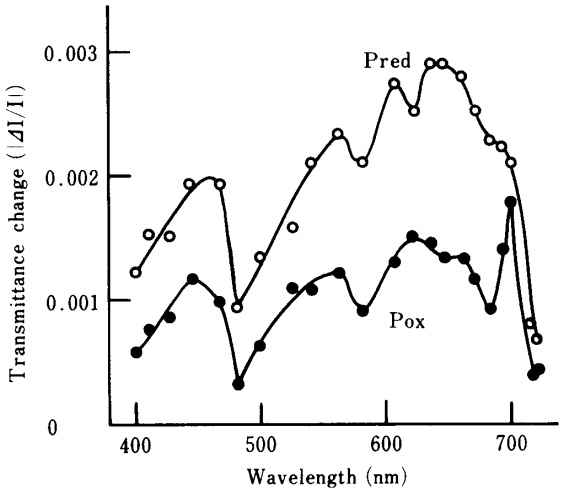


Fig. 6. Action spectra of P700 oxidation (Pox) and P700 reduction (Pred) in a kidney bean leaf.

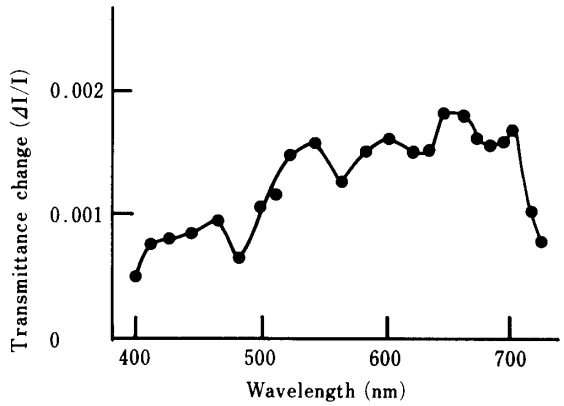


Fig. 7. Action spectrum of electrochromic shift (E) in a kidney bean leaf.

by Kok and Gott[13].

Remarkable differences in the action spectra of both systems were observed at wavelengths longer than 630 nm (Fig. 6). The effectiveness of photosystem I excitation by the flash light at 700 nm was high, while that of photosystem II was reduced to two thirds of the maximum value. A similar decrease of the effectiveness of P700 reduction above 650 nm was reported by Kok and Gott[13] with the chlorella cell free system. The low reactivity of the photosystems to light at around 480 nm, which corresponds to the absorption peak of carotenoid, was ascribed to the low efficiency of this pigment by Emerson and Lewis[4] and Inada[9].

The action spectrum of electrochromic shift differed from those of CO_2 fixation by intact leaves reported by Inada[8] and McCree[15] in which the values were not maintained at around 700 nm. In this regard, the action

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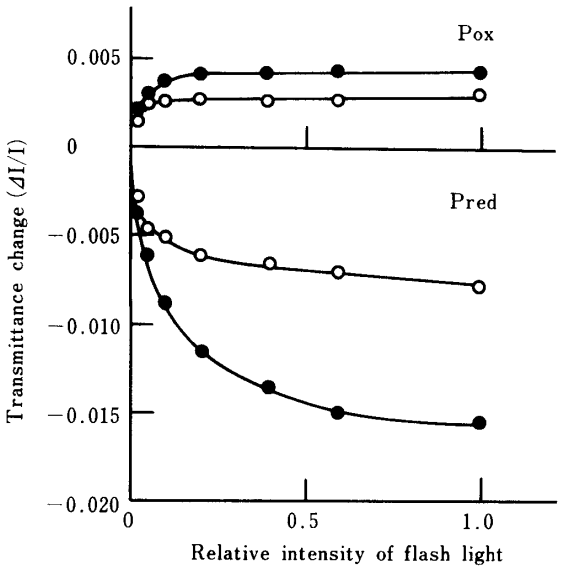


Fig. 8. Dependency of P700 oxidation (Pox) and P700 reduction (Pred) in kidney bean leaves upon the intensity of actinic flash light.

Leaves containing 30 nmol (— ○ —) and 51 nmol (— ● —) chlorophyll per cm^2 leaf area were used for the measurements. The intensity of the actinic flash of 1.0 was 6.5 $J \cdot m^{-2}$ per flash.

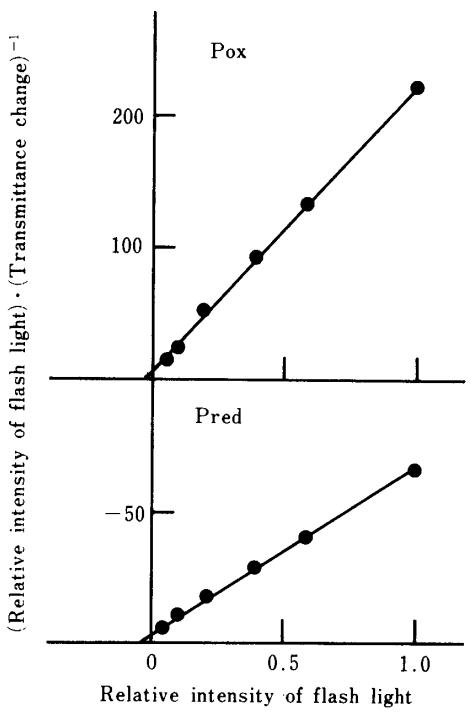


Fig. 9. Hofstee plots of P700 oxidation (Pox) and P700 reduction (Pred).

The results of the leaf containing 51 nmol chlorophyll per cm^2 leaf area in Fig. 8 are plotted.

spectrum of the electrochromic shift may not necessarily parallel that of CO_2 fixation. CO_2 fixation requires the synergy of the two photosystems while the induction of the electrochromic shift requires the excitation of photosystem I or photosystem II[22], though the participation of either photosystem is considered to be essential as the blockage of both photosystems by DCMU in the presence of strong detecting light caused a complete inhibition of the electrochromic shift.

Considering that the wavelength of the absorption maximum in intact leaves is near 680 nm, the effectiveness of light at 683 nm for the electrochromic shift was relatively low compared to the zone around this region (Fig. 7). It is considered that most of the flash light was absorbed by chloroplasts located at the surface of the leaf tissues and could not reach the chloroplasts located deep in the tissues because the intensities of the flash lights were decreased for the measurement of action spectrum while the absorption efficiency by the pigment was high around these wavelengths. This phenomenon may result in the decrease of the amount of reaction centers which were excited, as mentioned by Kok and Gott[13] and induced a screening and self absorption effect. Leaves are considered to be organized to collect effectively energy of natural light showing wide and gently sloped spectrum at the expense of the efficiency of the photoreactions at the maximum absorption region of photosynthetic pigments. On the other hand, low efficiency at blue light region around 480 nm was guessed to have relation to blue light responses in plant growth. Further investigations are needed to interpret the mechanism underlying the regulation of photoreactions by the leaf *per se*.

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