Biochimica et Biophysica Acta, 440 (1976) 337–345

BBA 47138

A DEMONSTRATION OF ENERGY TRANSFER FROM PHOTOSYSTEM II TO PHOTOSYSTEM I IN CHLOROPLASTS

K. SATOH, R. STRASSER and W. L. BUTLER

Department of Biology, University of California, San Diego, La Jolla, Calif. 92093 (U.S.A.)

(Received March 1st, 1976)

SUMMARY

Photosystem I activity of Tris-washed chloroplasts was measured at room temperature as the rate of photoreduction of NADP and as the rate of oxygen uptake mediated by methyl viologen in both cases using dichlorophenolindophenol plus ascorbate as the source of electrons for Photosystem I. With both assay systems the rate of electron transport by Photosystem I was stimulated approx. 20 % by the addition of 3-(3,4-dichlorophenyl)-1,1-dimethylurea which caused the Photosystem II reaction centers to close. Photosystem I activity of chloroplasts was measured at low temperature as the rate of photooxidation of *P*-700. Chloroplasts suspended in the presence of hydroxylamine and 3-(3,4-dichlorophenyl)-1,1-dimethylurea were frozen to −196 °C after adaptation to darkness or after a preillumination at room temperature. The Photosystem II reaction centers of the frozen dark-adapted sample were all open; those of the preilluminated sample were all closed. The rate of photooxidation of *P*-700 at −196 °C with the preilluminated sample was approx. 25 % faster than with the dark-adapted sample. We conclude from both the room temperature and the low temperature experiments that there is greater energy transfer from Photosystem II to Photosystem I when the Photosystem II reaction centers are closed and that these results are a direct demonstration of spillover.

INTRODUCTION

Our model of the photosynthetic apparatus presented previously [1, 2] incorporated two mechanisms to control the distribution of excitation energy. Incoming quanta were partitioned initially between Photosystem I and Photosystem II according to their relative cross-sections, the fraction going to Photosystem I being specified as α and that going to Photosystem II as β where Photosystem I and Photosystem II parameters were defined so that $\alpha+\beta = 1$. In addition, it was proposed that some of the excitation energy distributed initially to Photosystem II could be transferred subsequently to Photosystem I, a process which has been dubbed "spillover" in the literature. According to the model the yield of such energy transfer, $\varphi_{T(II\rightarrow I)}$, should depend on the rate constant for energy transfer, $k_{T(II\rightarrow I)}$, and state of the Photosystem II reaction centers. ($\varphi_{T(II\rightarrow I)}$ varies from a minimum value when the Photosystem II

reaction centers are all open to a maximum value when they are closed in the same manner that the yield of fluorescence from Photosystem II varies.) The model was tested to determine if it would account for the effects of divalent cations on energy distribution in chloroplasts [2]. According to that analysis the absence of divalent cations caused a redistribution of excitation energy into Photosystem I by increasing α approx. 20 % and $k_{T(II \to I)}$ approx. 90 %.

However, the conclusions as to energy transfer from Photosystem II to Photosystem I depended strongly on two assumptions. One was that the fluorescence of variable yield at any wavelength of emission was due solely to the photochemical activity of Photosystem II. That assumption was verified by showing that photochemical activity of Photosystem I at −196 °C (i.e. the photooxidation of *P*-700) did not result in fluorescence yield changes at any wavelength of emission and by the finding that the kinetic behavior of the fluorescence of variable yield at 730 nm was precisely the same as that at 690 nm [2]. The second assumption was that fluorescence at 730 nm at −196 °C emanated only from Photosystem I units, i.e. that any long wavelength tail of Photosystem II fluorescence at 730 nm could be neglected in comparison to the 730 nm emission from Photosystem I. Emission spectra of purified samples of the light-harvesting chlorophyll *a*/*b*-protein [2] and of isolated Photosystem II complexes [3] confirmed that the 730 nm fluorescence from these sources at −196 °C could be considered to be negligible compared to the 730 nm emission from Photosystem I particles, provided the emission spectra of the isolated samples were representative of their emission spectra in vivo. However, the possibility has not been excluded that the fluorescence from Photosystem II in chloroplasts does have an appreciable emission band at 730 nm and that this component is lost when the chlorophyll LH or Photosystem II complexes are purified*. If that were the case the fluorescence of variable yield measured at 730 nm could be due to direct emission from Photosystem II rather than to energy transfer from Photosystem II to Photosystem I, and energy distribution could be controlled solely by the initial partitioning of excitation energy. The question we address in the present work is whether the distribution of excitation energy is also determined by energy transfer from Photosystem II to Photosystem I, i.e. if the rate constant for spillover, $k_{T(II \to I)}$ is a significant parameter.

The model suggests experiments which should verify spillover of excitation energy from Photosystem II to Photosystem I if such a process is significant. The yield of such energy transfer should increase as the Photosystem II reaction centers close so that the yield of photochemistry of Photosystem I should also increase. We have examined that proposition by measuring the rate of Photosystem I activity in Tris-washed chloroplasts at room temperature in the absence and presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). We also measured the rate of photooxidation of *P*-700 in chloroplasts at −196 °C under conditions where the Photosystem II reaction centers were either all open or all closed. According to the

* The possibility of such changes in the emission spectrum due to the isolation procedures gained more credence when we found that the low temperature emission spectrum of the purified *P*-700-chlorophyll *a*-protein described by Shiozawa et al. [4] showed a single, strong emission band at about 685 nm with very little emission at 730 nm (very similar to the emission spectrum of the purified chlorophyll *a*/*b* protein complex shown in ref. 2) even though the Photosystem I particles from which the *P*-700-chlorophyll *a*-protein was purified showed at −196 °C a single dominant emission band at 730 nm with very little emission at 690 nm.

model, if spillover does occur, Photosystem I activity should be greater when the Photosystem II reaction centers are closed.

METHODS

Tris-washed chloroplasts were prepared from spinach leaves by methods described previously [5]. Ferredoxin and ferredoxin-NADP oxidoreductase were also prepared from spinach leaves by methods described previously [6].

The reaction medium for the photoreduction of NADP contained Tris-washed chloroplasts with 50 μg of chlorophyll, 155 μmol of Tris · HCl, 150 μmol NaCl, 20 μmol sucrose, 15 μmol sodium ascorbate, 0.2 μmol DPIP (2,6-dichlorophenolindophenol), 1 μmol NADP and an excess of ferredoxin and ferredoxin-NADP oxidoreductase in a volume of 3.6 ml (pH 7.8). DCMU was added as 0.1 ml of a 370 μM ethanolic solution to give a final concentration of 10 μM; the control samples contained 0.1 ml of ethanol. The photoreduction of NADP was followed in a 1 cm quartz cuvette by measuring the absorbance at 340 nm as a function of time with a Cary 17 spectrophotometer. The sample compartment had a port which permitted the sample to be irradiated with actinic light. The actinic source was either a Spectra-Physics model 133 Ne-He laser with a 634 nm interference filter giving an intensity of 300 μW/cm^2 of red light or a tungsten lamp with two Calflex heat-reflecting filters, a Toshiba V-R69 cut-off filter and a 705 nm interference filter giving 2.2 mW/cm^2 of far red light. The red light was used at a relatively low intensity so that the rates of photoreduction of NADP in the red and far red light would be approximately the same. The rate of NADP photoreduction which was 10–20 % of the saturation rate at these intensities was linearly dependent on light intensity. A Corning ultraviolet filter 5840 protected the 1P28 photomultiplier tube from the scattered actinic light.

The reaction medium for the oxygen uptake measurements contained Tris-washed chloroplasts with 50 μg of chlorophyll, 250 μmol of Tris · HCl, 20 μmol sucrose, 80 μmol sodium ascorbate, 0.4 μmol DPIP and 0.8 μmol methyl viologen in a total volume of 4.9 ml (pH 7.8). 0.1 ml of a 0.5 mM ethanolic solution of DCMU (final concentration 10 μM) or 0.1 ml of ethanol were also added during the course of the experiment. Oxygen uptake was measured polarographically with a Clark-type oxygen electrode. Actinic light was obtained with a tungsten lamp and two Calflex heat-reflecting filters with either a Balzers 658 nm interference filter for red light or two Corning filters, 9780 and 9782, for blue light. Typically actinic light intensities were 3.5 mW/cm^2 in the red or 2.0 mW/cm^2 in the blue.

The reaction medium for the fluorescence measurements was the same as that used for the photoreduction of NADP. Fluorescence was measured at 685 nm with an EMI 9558 photomultiplier tube with a Balzers 685 nm interference filter and two Toshiba V-R68 cut-off filters. The fluorescence was excited by the Ne-He laser system (300 μW/cm^2) used for the photoreduction of NADP.

The chloroplasts which were used for the measurements of the rate of photooxidation of *P*-700 were prepared from spinach leaves which had been preilluminated with white light (900 μW/cm^2) for 10 min just before the chloroplasts were prepared. The reaction medium for these experiments contained chloroplasts with 600 μg chlorophyll, 200 μmol of Tris · HCl, 2 μmol NaCl, 80 μmol sucrose, 80 μmol sodium ascorbate, 100 μmol NH_4OH and 0.04 μmol DCMU (10 μM), pH 7.8, in a total

volume of 4.0 ml. 0.3-ml aliquots, giving a sample depth of 2 mm, were frozen to liquid nitrogen temperature in our vertical cuvette and Dewar system [7]. The control sample was kept in complete darkness prior to freezing. The preilluminated sample was irradiated with blue light (5 mW/cm^2) for 30 s at room temperature and returned to darkness for 30 s at room temperature before freezing. The frozen samples were irradiated during the measurement by a repetitive series of xenon flashes (0.3 μs) from a General Radio Stroboslave, type 1539, at a rate of 1 per s. The photooxidation of *P*-700 caused by the flashes was monitored continuously with a weak 703 nm measuring beam from a monochromator. The light flashes and the measuring beam were delivered to the window on the bottom of the Dewar by two fiber-optic light pipes which gave uniform illumination in the bottom of the cuvette. The light transmitted through the sample (or fluoresced by the sample) was measured with red-sensitive side window phototube protected by a red cut-off filter and a 703 nm interference filter. The signal from the phototube was recorded with a fast (0.1 s time constant) Hewlett Packard X-Y recorder model 7047A with the *x*-axis being time. The recorder pen was driven off scale by the photosignal during the flash but returned rapidly to indicate a valid transmission reading of the 703 nm measuring beam between flashes. The data presented in Fig. 4 are photocopies of the original recordings.

RESULTS AND DISCUSSION

Photosystem I activity of Tris-washed chloroplasts was measured as the rate of photoreduction of NADP with DPIP and ascorbate as an electron donor system. Measurements were made with pairs of samples, one in the presence of 10 μM DCMU, the other with an aliquot of ethanol. The results of two such experiments measured in the absence and presence of 5 mM Mg^{2+} are shown in Figs. 1A and 1B. Relative rates

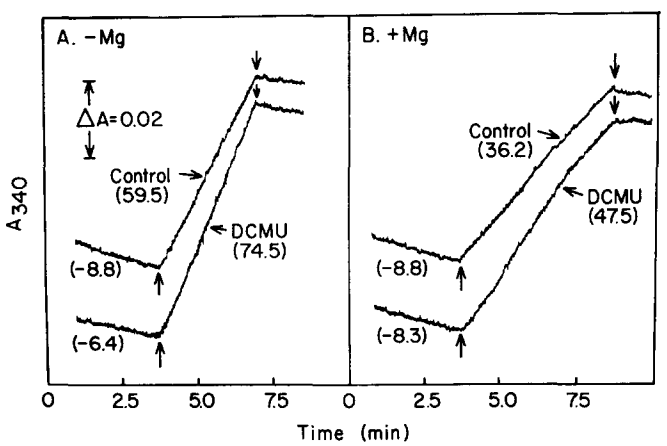

Fig. 1. Photoreduction of NADP by Tris-washed chloroplasts (13.5 μg chlorophyll/ml). The reaction medium contained ferredoxin and ferredoxin-NADP oxidoreductase to mediate electron transport from Photosystem I to NADP and DPIP plus ascorbate to supply electrons to Photosystem I. NADPH is measured by the absorbance at 340 nm. Red light (633 nm) of 300 μW/cm^2 is on at the upward arrows, off at the downward arrows. The curves marked DCMU indicate the results obtained in the presence of 10 μM DCMU. The controls contain an aliquot of ethanol. The figures in parentheses indicate relative slopes. (A) In the absence of Mg^{2+}. (B) In the presence of 5 mM Mg^{2+}.

TABLE I

RATE OF PHOTOREDUCTION OF NADP BY TRIS-WASHED CHLOROPLASTS IN RED AND FAR RED LIGHT

Measurements were made in the absence and presence of 10 μM DCMU and in the absence and presence of 5 mM Mg^{2+}. Rate of NADP photoreduction is given as μmol NADP/mg chlorophyll per h.

Actinic light	Mg^{2+}	Rate of NADP photoreduction		Stimulation (%)
		−DCMU	+DCMU	
Red*	−	12.6	15.3	21.4
Red*	+	9.4	11.4	21.3
Far red**	−	11.0	10.8	−1.8

* 633 nm, 300 μW/cm^2.
** 705 nm, 2.2 mW/cm^2.

of the absorbance change at 340 nm are indicated by the figures in parentheses. After correcting the rate obtained in the light for that obtained during the previous dark period, the results of Fig. 1A indicate that DCMU stimulated the rate of photoreduction of NADP 18 % and the results of Fig. 1B, a stimulation of 24 %. These experiments invariably showed a stimulation of Photosystem I activity by DCMU but the extent of the stimulation was variable. A series of 11 experiments measured in the absence of Mg^{2+} showed stimulations ranging from 7.5 to 24.9 % giving an average value of 16.9±6.5 % (the uncertainty figures are calculated as standard deviations). In a similar series of 12 experiments in the presence of Mg^{2+} the values of the stimulation ranged from 5.9 to 28.3 % giving an average stimulation of 18.0± 6.4 %. We did not find in these experiments that the DCMU stimulation of Photosystem I activity was influenced significantly by the presence or absence of Mg^{2+}. The stimulation of Photosystem I activity in Tris-washed chloroplasts was obtained only when the actinic light was absorbed by Photosystem II as well as by Photosystem I. Table I compares results obtained with red and far red light with the light intensities adjusted to give comparable rates of photoreduction of NADP. It is apparent that DCMU stimulates the rate of photoreduction of NADP in red light but not in far red light.

Fluorescence yield measurements with Tris-washed chloroplasts made under the same conditions that were used to measure the photoreduction of NADP are shown in Fig. 2. These results were obtained in the presence of ferredoxin, ferredoxin-NADP oxidoreductase, and NADP as the electron acceptor system and DPIP plus ascorbate as the electron donor system but essentially the same results were obtained in the absence of the electron donor and acceptor systems. The definition of F_0 was only approximate in these measurements. In the presence of actinic light the fluorescence yield increased to a level indicating that 50–60 % of the Photosystem II reaction centers were closed at the steady state in the light. On the addition of DCMU during irradiation all of the Photosystem II reaction centers close and the fluorescence increases to the F_M level. We attribute the stimulation of Photosystem I activity by DCMU to the increased yield of energy transfer from Photosystem II to Photosystem I which should occur when the Photosystem II reaction centers close.

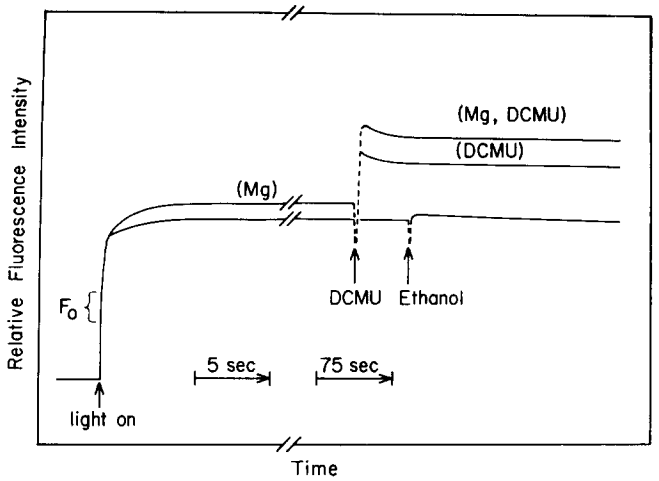

Fig. 2. Relative intensity of fluorescence of Tris-washed chloroplasts at 685 nm. The reaction medium and chlorophyll concentration are the same as in Fig. 1. Red light (633 nm) of 300 μW/cm^2 is turned on at the initial part of the trace; DCMU and ethanol were added where indicated. Measurements were made in the presence and absence of 5 mM Mg^{2+}.

Photosystem I activity was also measured as the rate of oxygen uptake mediated by methyl viologen with DPIP plus ascorbate as the electron donor. In these experiments, DCMU could be added to the sample during the course of the experiments. Typical results showing the effect of adding DCMU or an aliquot of ethanol are shown in Fig. 3. The upward step in the oxygen electrode trace at the point of addition of

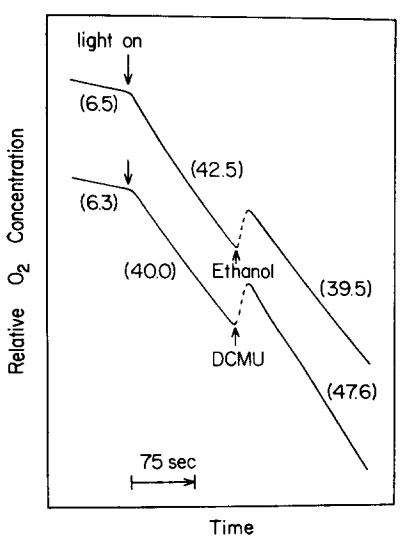

Fig. 3. Oxygen uptake by Tris-washed chloroplasts (10 μg/ml) mediated by methyl viologen with DPIP plus ascorbate as the electron donor system. Blue light (2.0 mW/cm^2) on at the downward arrows. DCMU and ethanol were added where indicated. The figures in parentheses indicate relative slopes.

DCMU and ethanol is due to the oxygen present in these aliquots. The data in Fig. 3 indicate a stimulation in the rate of oxygen uptake of 22 % by DCMU and an inhibition of 8 % by the ethanol. In a series of 17 experiments the addition of 10 μM DCMU stimulated the rate of oxygen uptake 21.6±9.7 % (individual values of stimulation ranged from 9.5 to 42.6 %); in a series of 10 experiments ethanol inhibited the rate 6.8±4.5 % (values ranged from 0 to 14.1 %).

A demonstration of spillover was also sought in measurements of the rate of photooxidation of *P*-700 at −196 °C. In these experiments chloroplasts were suspended in the presence of hydroxylamine and DCMU and frozen to −196 °C. The control chloroplasts were kept in complete darkness until the measurements at −196 °C began; the preilluminated chloroplasts were illuminated at room temperature for 30 s with blue light and kept for another 30 s in the dark before freezing to −196 °C. It has been shown previously [8, 9] that irradiation of chloroplasts at room temperature in the presence of DCMU and hydroxylamine causes the primary electron acceptor of Photosystem II, as monitored by fluorescence yield or by *C*-550 absorbance, to go reduced and to remain reduced in the dark after the irradiation and that this condition can be frozen in ref. 9. A dark period at the end of such an irradiation allows *P*-700 to return to the fully reduced state before freezing. Thus, in the two conditions just described, *P*-700 is fully reduced but the Photosystem II reaction centers are either all open (dark-adapted sample) or all closed (preilluminated sample).

The results of a typical experiment are shown in Fig. 4. The transmission of the sample was monitored with a weak measuring beam at 703 nm in the presence of brief (3 μs) flashes from a xenon arc at the rate of 1 per s. During the flash the recorder pen goes off scale due to transmission of the flash and fluorescence from the sample but recovers rapidly to indicate the true transmission of the measuring beam between flashes. The lower envelope of the flash profile indicates the rate of photooxidation of *P*-700 due to the repetitive flashes. Complete transformation of *P*-700 is indicated by the level at which the recording starts across the chart a second time. The phototransformation of *P*-700 at −196 °C plots as a second-order reaction at least out to the 75 % transformation point but we draw no conclusions from the order of these kinetics. The absorbance of the sample to the spectral distribution of the flash will cause the actinic intensities to vary markedly as a function of the distance within the sample so that the true kinetic behavior of the photoreaction will be altered. Nevertheless, having straight line second-order plots facilitates the determination of relative rate constants. The points at which the photoconversion of *P*-700 is 1/4, 1/3, 1/2, 2/3 and 3/4 complete are indicated on the rate curves and these values are used for the second-order plots in the lower part of Fig. 4 (for such small values of ΔT, ΔT is proportional to the amount of *P*-700 transformed at any time t). The slopes of the second-order plots are taken to be proportional to the rate of photooxidation of *P*-700. The data in Fig. 4 indicate that the rate of phototransformation of *P*-700 is 22 % faster for the preilluminated sample. Measurements with different batches of chloroplasts made on different days gave a range of results of which the data presented in Fig. 4 are fairly typical. The average of five experiments on a typical day gave values of 0.75±0.18 for the dark-adapted samples and 0.94±0.16 for the preilluminated samples for an average stimulation of 25 %. On another day, the average of five experiments gave values of 0.69±0.20 and 1.14±0.26 for the dark-adapted and pre-

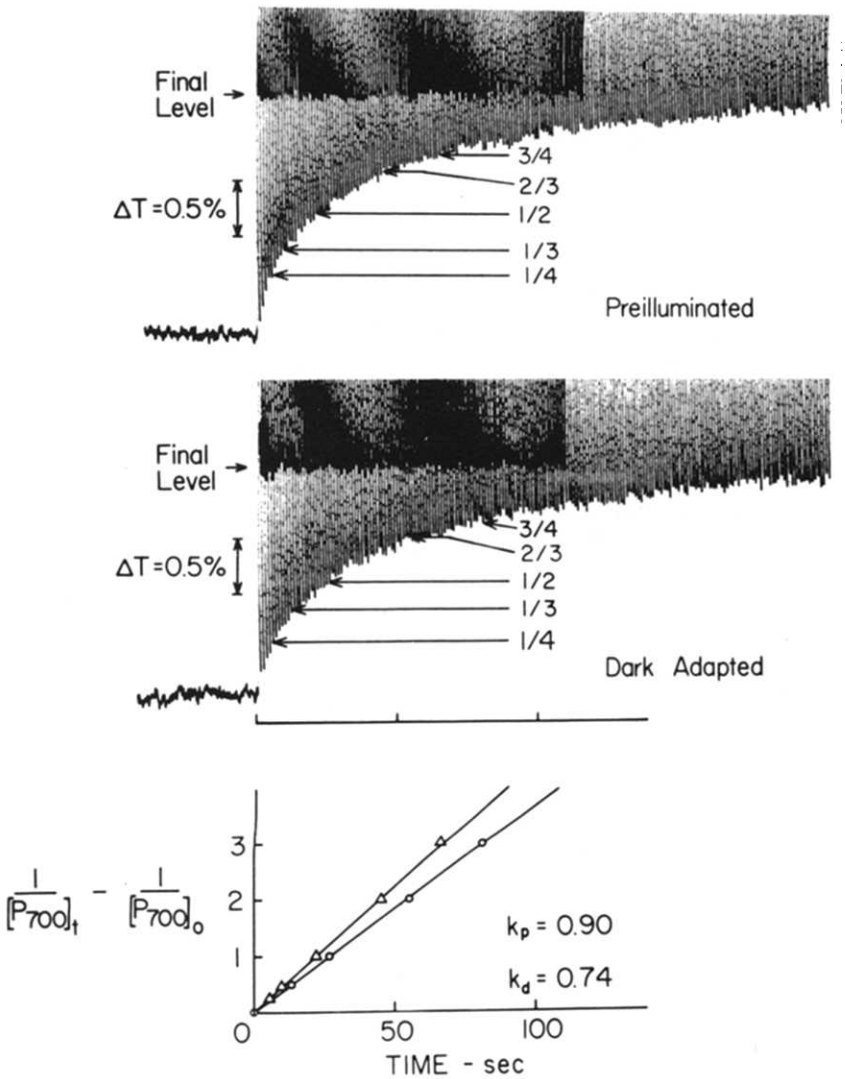

Fig. 4. The photooxidation of *P*-700 in chloroplasts at −196 °C. Chloroplasts suspended in the presence of 10 μM DCMU and 25 mM NH_4OH were frozen to −196 °C after complete darkness (dark-adapted sample) or after a 30 s irradiation with blue light and a subsequent 30 s dark period at room temperature just before freezing to −196 °C. The transmission of the sample at 703 nm was monitored as a single-beam measurement. Flashes at the rate of 1 per s were superimposed on the measuring beam starting at zero time. The flash drives the recorder pen off scale but the pen recovers between flashes. The flashes transform *P*-700 causing the transmission to increase as indicated by the lower envelope of the flash profiles. The rates of transformation of *P*-700 for the preilluminated and dark-adapted samples are plotted as a second-order reaction in the lower part of the figure. The relative second-order rate constants are 0.90 for the preilluminated sample and 0.74 for the dark-adapted sample.

illuminated samples for an average stimulation of 65 %. On other days, however, the chloroplasts showed an average stimulation of 0 %. We do not know all of the factors involved in this variability but we appeared to obtain consistently higher values of stimulation when we irradiated the spinach leaves for a short period just before the chloroplasts were prepared.

CONCLUSION

We take these results showing that Photosystem I activity is enhanced when the Photosystem II reaction centers are closed to indicate that energy transfer from Photosystem II to Photosystem I is a significant process. We also take these results to represent a further confirmation of our model and the original supposition that the fluorescence of variable yield at 730 nm is an index of energy transfer from Photosystem II to Photosystem I.

ACKNOWLEDGEMENTS

This work was supported by a National Science Foundation Grant, BMS73-06884. We are grateful to Drs. J. P. Thornber and R. S. Alberte for a purified sample of the *P*-700-chlorophyll *a*-protein.

REFERENCES

1 Butler, W. L. and Kitajima, M. (1975) Proceedings of the Third International Congress on Photosynthesis (Avron, M., ed.), pp. 13–24, Elsevier Scientific Publ. Co., Amsterdam
2 Butler, W. L. and Kitajima, M. (1975) Biochim. Biophys. Acta 396, 72–85
3 Wessels, J. S. C., Van Alphen-Van Waveren, O. and Voorn, G. (1973) Biochim. Biophys. Acta 292, 741–752
4 Shiozawa, J. A., Alberte, R. S. and Thornber, J. P. (1974) Arch. Biochem. Biophys. 165, 388–397
5 Yamashita, T. and Butler, W. L. (1968) Plant Physiol. 43, 1978–1986
6 Huzisige, H., Satoh, K., Tanaka, K. and Hayashida, T. (1963) Plant Cell Physiol. 4, 307–322
7 Butler, W. L. (1972) Methods in Enzymology (San Pietro, A., ed.), Vol. 24B, pp. 3–25, Academic Press, New York
8 Bennoun, P. (1970) Biochim. Biophys. Acta 216, 357–363
9 Erixon, K. and Butler, W. L. (1971) Biochim. Biophys. Acta 234, 381–389