Biochimica et Biophysica Acta, 934 (1988) 144–150
Elsevier

BBA 42780

Influence of electrostatic screening by cations on energy coupling between Photosystem II reaction centres and the light-harvesting chlorophyll *a/b* protein complex II

Giuseppe Zucchelli, Flavio M. Garlaschi and Robert C. Jennings

Centro C.N.R. Biologia Cellulare e Molecolare delle Piante, Dipartimento di Biologia, Università di Milano, Milano (Italy)

(Received 7 December 1987)

Key words: Light harvesting chlorophyll *a/b* complex; Cation; Photosystem II; Energy transfer; Chlorophyll fluorescence; (Spinach chloroplast)

In the present paper the increased F_0 fluorescence emission encountered upon lowering the cation concentration below saturating levels is studied. Comparison of the F_0 fluorescence excitation spectra at different cation concentration with that of chlorina barley mutant which lacks LHCP II and also with phosphorylated spinach chloroplasts indicates that the increased F_0 fluorescence originates from LHCP II which becomes partially uncoupled from the Photosystem II reaction centre at low cation concentration. Studies with cations of different valency suggest that this phenomenon is controlled by membrane surface electrostatic charges in a similar way to thylakoid stacking and the 'spill-over'-type interactions between the two photosystems. Cation titration experiments of phosphorylated chloroplasts suggest that both the 'mobile' and the 'non-mobile' LHCP II display a cation-sensitive energy transfer to Photosystem II reaction centres.

Introduction

It is well known that cations are of paramount importance in mantaining the characteristic granal structure of higher plant chloroplasts [1–4] and the associated lateral separation of the two photosystems with their respective antenna complexes [5]. This lateral separation of the two photosystems, with PS II and its LHCP II antenna concentrated in the appressed membrane regions of the grana and PS I located in the nonappressed membranes, is thought to be essentially responsible for the low-energy transfer between PS II and PS I and high-energy transfer between PS II units in the presence of cations. Thus cations are envisaged to modify the energy-transfer pathways between the photosystems by large-scale distance effects. Little attention has been given to a possible cation role in influencing energy transfer within each photosystem-antenna complex, though several authors have proposed that cations may promote a tighter energy coupling between PS II and its LHCP II antenna complexes [6–8]. However, direct evidence for this is lacking.

Hipkins [9] was the first to notice that when the Mg ion concentration is lowered below saturating levels the nonvariable fluorescence (F_0) increases. This observation was subsequently confirmed in

Abbreviations: F_0, nonvariable fluorescence; F_M, maximal fluorescence; PS I, Photosystem I; PS II, Photosystem II; LHCP II, light-harvesting chlorophyll *a/b* protein complex II; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; TEC, tris-(ethylenediamine)cobalt(III)chloride.

Correspondence: G. Zucchelli, Centro C.N.R. Biologia Cellulare e Moleculare delle Piante, Dipartimento di Biologia, Università di Milano, via Celoria 26, 20133 Milano, Italy.

our laboratory [10] and tentatively attributed to an effect of Mg ions on the rate constant of energy transfer to PS II reaction centres. In the present paper we present further studies on the increased F_0 fluorescence emission at subsaturating cation concentrations. It is concluded that the proposed decreased energy transfer from the PS II antenna to its reaction centre is in fact brought about by decreased LHCP II-PS II energy coupling. This decreased coupling involves surface charge screening effects. Both the 'mobile' and 'nonmobile' LHCP II subpopulations [11,12] seem to display cation-sensitive interactions with the PS II reaction centres.

Materials and Methods

Chloroplasts were extracted from freshly harvested spinach or barley leaves by blending in 30 mM Tricine (pH 8), 0.4 M sucrose, 10 mM NaCl and 5 mM $MgCl_2$ (total Na^+ concentration: 25 mN). After filtering through cheese cloth they were pelleted by a brief centrifugation at $1500 \times g$ and resuspended in the above medium minus sucrose. An equal volume of the sucrose containing medium was then added and the chloroplasts pelleted again at $1500 \times g$, to be subsequently resuspended in a medium similar to the one described above but with sucrose 0.2 M and the appropriate cation concentration, depending on the experiment. Storage in ice and in darkness was for at least 1 h, at which time measurements were started. The reaction medium was identical to the storage medium and chlorophyll concentration was $4\ \mu g \cdot ml^{-1}$.

The F_0 and F_M fluorescence levels were determined in the assembly previously described [4]. The excitation light was filtered across a Corning 4-96 filter and emission was measured at 684 nm (Balzers interference filter; half-peak bandwidth, 10 nm). 25 μM DCMU was added after the F_0 had been measured to determine the F_M value.

Fluorescence excitation spectra between 400 and 600 nm were measured in an SLM 4800 spectrofluorimeter with the emission wavelength set at 682 nm. When excitation spectra were measured near the F_0 level an extremely weak measuring beam was employed and the chloroplast suspension was continuously stirred in the presence of an electron acceptor (0.1 mM methyl viologen). Under these conditions the fluorescence level attained was that of the plateau of the fast induction phase. For measurement of spectra at the F_M level DCMU (25 μM) was added. Ratio spectra were normalised at 440 nm.

p-Phenylenediamine reduction was measured at 25 °C and at low light intensity ($10\ W \cdot m^{-2}$, Corning 4-96 filter) with a Clark-type oxygen electrode, in the presence of 0.3 mM *p*-phenylenediamine and 1.3 mM potassium ferricyanide.

Phosphorylation of spinach thylakoid membranes was achieved by kinase activation in the dark with the NADPH/ferredoxin system in the presence of $MgCl_2$ (5 mM) for 10 min, as previously described [13].

Titration of the chloroplast fluorescence with the artificial quenchers *m*-dinitrobenzene and dibromothymoquinone at the F_0 fluorescence level was performed by incubating the chloroplasts for 3 min at the desired quencher concentration before F_0 determination.

Results

The influence of cations on the F_M fluorescence level of isolated chloroplasts has been demonstrated to be mediated by non-specific electrostatic interactions of the Gouy-Chapman type by titration experiments with cations of different valency [14]. We therefore titrated the F_M and F_0 fluorescence levels with monovalent (NaCl), divalent ($MgCl_2$) and trivalent (TEC) cations to gain informations on the nature of the cation effect on F_0. Fig. 1 shows the results of these experiments. In all cases at subsaturating concentrations the F_M fluorescence decreased greatly, due to the distance-determined increased energy flow from PS II to PS I (the so-called 'spill-over') as was already noted [14]. On the other hand, the F_0 fluorescence increased substantially upon lowering the concentration of all three cation types, to attain a maximum value and to decline subsequently. The increases in F_0 at low cation concentrations in these experiments were greater than those observed previously [9,10] and typically were more than 50% higher than at saturating cation concentrations. The similar shape of the F_0 titration curves with the three cations and the order of

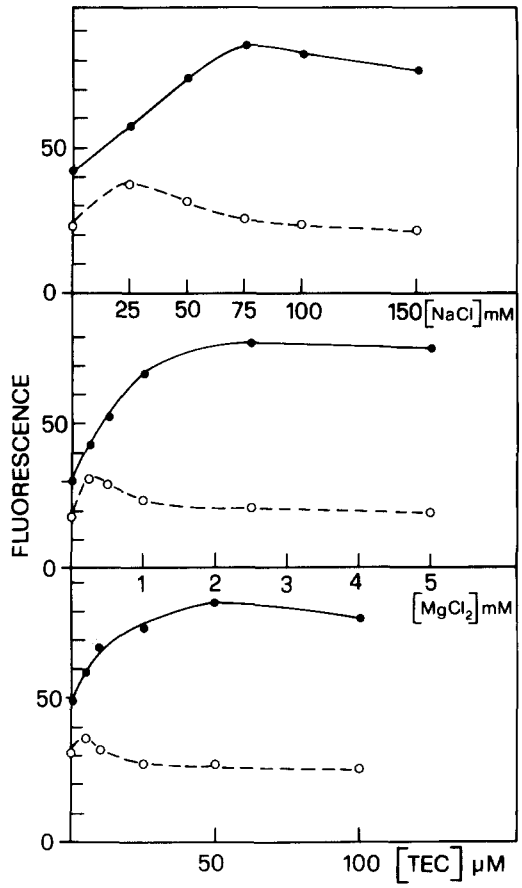

Fig. 1. Titration of the F_M and F_0 fluorescence levels with cations of different valency. Closed symbols: F_M; open symbols: F_0. Chloroplasts were preincubated for at least 1 h at the different concentrations of NaCl, $MgCl_2$ and tris(ethylenediamine)cobalt)III)chloride (TEC), prior to the measurements. Background concentration of Na^+ was 25 mN in all cases. Each data point is the average of six separate determinations, performed with two different chloroplast preparations.

effectiveness ($[C^{3+}] > [C^{2+}] > [C^+]$) suggest that also in this case cations affect the F_0 fluorescence level via nonspecific electrostatic interactions of the Gouy-Chapman type.

Recent analyses of the fluorescence decay components suggest that there is a small PS I emission at room temperature which may be proportionally greater at the F_0 level than at the F_M level [15–17]. Clearly a substantial PS I contribution to the F_0 fluorescence would complicate the interpretation of the F_0 changes as a function of cation concentration. To investigate this possibility we have measured the fluorescence excitation spectra at both the F_M and F_0 levels in the wavelength range 400–520 nm and determined the ratio of these two parameters (Fig. 2). It is well known that there are large differences in the relative optical cross section of PS I with respect to PS II in this wavelength range (e.g., Refs. 18 and 6), mainly due to the relatively minor contribution of LHCP II absorption to PS I. The peak of relative PS II action with respect to PS I is at 480 nm where the PS II/PS I optical cross-section ratio seems to be about 50–70% higher than at 400–440 nm [18,6]. We have confirmed this result with our chloroplast preparations (unpublished data). Thus, if the PS I contribution to the F_0 fluorescence were large with respect to that at the F_M level, we would expect to observe a minimum in the F_0/F_M ratio spectrum near 480 nm. This, however, was not observed (Fig. 2). The F_0/F_M is almost constant between 400 and 520 nm, thus suggesting that PS I does not make a significantly greater contribution to the F_0 fluorescence than to that at the F_M level, which is generally accepted as being small.

We have next examined the fluorescence excitation spectra in the wavelength range 400–600 nm at the F_0 level in the presence of Mg ions (5 and 0.25 mM). The data are presented in Fig. 3a as the ratio of these two spectra and thus represent the spectrum of the relative changes of fluorescence emission at 5 mM Mg^{2+} with respect to 0.25 mM. This ratio spectrum is fairly constant from 400 nm to 440 nm and shows a significant decrease for wavelengths above 440 nm. This decreased relative fluorescence has three broad minima near 483, 520 and 555 nm and a pronounced shoulder at 455

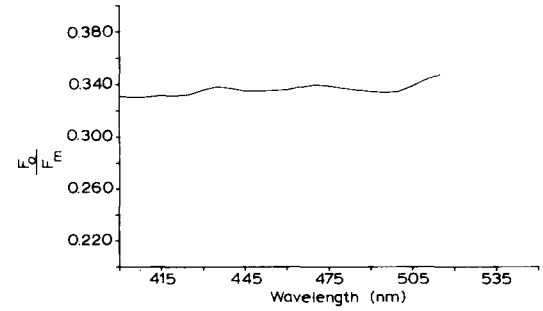

Fig. 2. The ratio of the F_0 and F_M fluorescence induction parameters for excitation wavelengths between 400 and 520 nm. Measurements were performed with chloroplasts suspended in the presence of 5 mM $MgCl_2$.

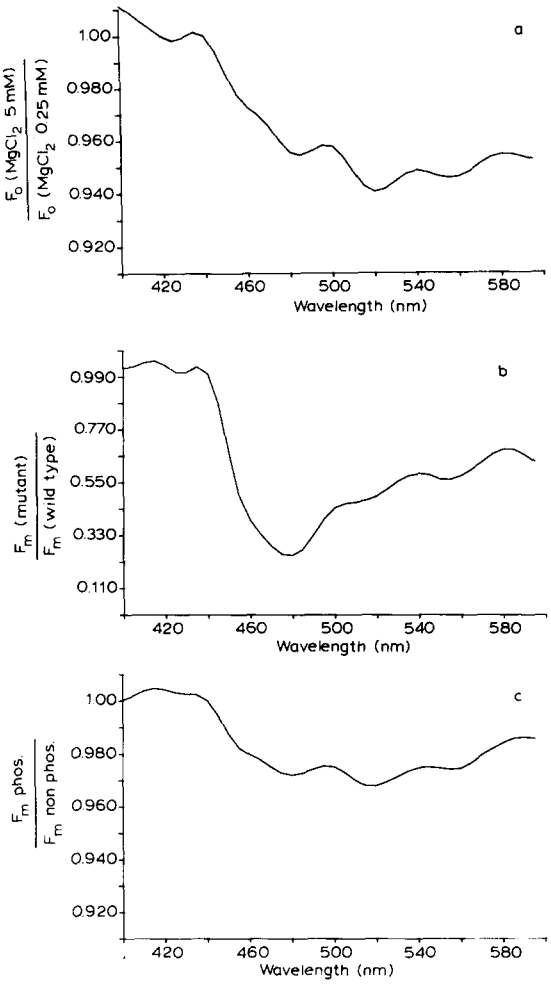

Fig. 3. The ratio of the fluorescence excitation spectra of: (A) spinach chloroplasts suspended in the presence of 5 mM and 0.25 mM $MgCl_2$ at the F_0 fluorescence level; (B) barley chloroplasts prepared from the chlorina mutant and wild type at the F_M level; (C) of phosphorylated and nonphosporylated spinach chloroplasts at the F_M level. Chloroplasts of (B) and (C) were resuspended in the presence of 5 mM $MgCl_2$.

nm. In an attempt to understand the significance of this ratio spectrum, we also examined the analogous ratio excitation spectra of: (a) the chlorina barley mutant lacking LHCP II with respect to the wild type (Fig. 3b) and (b) spinach chloroplasts in which LHCP II was phosphorylated with respect to nonphosphorylated chloroplasts (Fig. 3c). In the case of the barley the shape of the ratio spectrum will be determined largely by the relative absorption cross-section of LHCP II with respect to the LHCP II-PS II matrix. In the case of the phosphorylated chloroplasts it is generally accepted that LHCP II phosphorylation leads to the movement of a 'mobile' population of LHCP II from the LHCP II-PS II to PS I where its fluorescence is quenched [11–13,19,20]. Thus the ratio spectrum of phosphorylated to nonphosphorylated chloroplasts should also be determined by the relative absorption cross section of LHCP II with respect to the total LHCP II-PS II matrix. In both cases the ratio of fluorescence excitation spectra is approximately constant in the wavelength range 400–440 nm, with broad minima at 480–483 nm, 516–520 nm and 555–562 nm and a pronounced shoulder at 455–459 nm. These spectra therefore resemble the ratio spectrum of chloroplasts suspended in 5 mM Mg^{2+} with respect to 0.25 mM Mg^{2+}. We therefore conclude that the increased F_0 fluorescence encountered upon lowering the concentration of the screening cation in the suspension medium is caused by a decrease in energy coupling between LHCP II and the PS II traps leading to increased fluorescence emission from LHCP II itself.

We have analysed the F_0 fluorescence increase measured upon reducing the Mg^{2+} concentration from 5 mM to 0.25 mM by the double fluorescence quencher titration technique [10,21]. Decreased energy coupling of LHCP II with the PS II traps, which leads to a substantial increase in the fluorescence emission from LHCP II itself, should lead to a macroscopically heterogeneous fluorescence emission as judged by this method [10,17,21,22]. In this case we would expect the 'quencher interaction index' ratio (the ratio of the titration slopes at Mg^{2+} concentration of 0.25 mM with respect to 5 mM) to be greater than the ratio of the corresponding F_0 values before quencher addition, due to the greater quenching efficiency of the artificial quencher in LHCP II partially uncoupled from PS II traps than in the rest of the PS II antenna which remains fully coupled to the reaction centres. In Table I data are presented for quencher titrations of F_0 fluorescence of chloroplasts suspended at 5 mM Mg^{2+} and at 0.25 mM Mg^{2+}. Dibromothymoquinone and *m*-dinitrobenzene were used as quenchers and it can be seen that in both cases the quencher interaction index ratio is greater than the ratio of the corresponding

TABLE I

THE DOUBLE QUENCHER TITRATION ANALYSIS OF THE F_0 FLUORESCENCE OF CHLOROPLASTS SUSPENDED IN THE PRESENCE OF 5 mM OR 0.25 mM $MgCl_2$

Fluorescence titrations were performed in separate experiments with dibromothymoquinone or *m*-dinitrobenzene; quencher additions were 0.7 and 150 μM, respectively. The data are presented as the ratio of measurements performed at 0.25 mM $MgCl_2$ with respect to 5 mM.

Quencher used	Quencher interaction index ratio	F_0 fluorescence ratio
Dibromothymoquinone	1.67	1.42
m-Dinitrobenzene	1.61	1.23

F_0 values measured before quencher addition. This indicates that the fluorescence increase at 0.25 mM Mg^{2+} is heterogeneous and is in agreement with the concept of LHCP II energy uncoupling.

Recent research suggests that LHCP II may consist of two distinct subpopulations, a potentially 'mobile' fraction which becomes physically detached from the LHCP II-PS II matrix following phosphorylation and an 'immobile' fraction which, even though becoming phosphorylated, remains coupled to PS II [11,12,23]. We have asked the question of whether the cation effect on LHCP II-PS II energy coupling involves only one of these two subpopulations or whether both are cation sensitive. To this end chloroplasts were subjected to phosphorylating conditions for 10 min, to maximise photosystem cross-section changes (LHCP II migration from PS II to PS I) without bringing about increased 'spill-over'-type interactions [23]. As we have previously observed that under these conditions significant changes in photosystems cross-section do not occur after 3–6 min of phosphorylation, despite continued phosphate incorporation into the two major LHCP II polypeptides, we assume that the bulk of 'mobile' LHCP II has migrated towards PS I. In Fig. 4 data are presented concerning the titration with Mg^{2+} of F_m and F_0 fluorescence levels of both phosphorylated and nonphosphorylated spinach chloroplasts. Apart from the commonly observed increase in the phosphorylation-induced F_M quenching encountered at subsaturating Mg^{2+} concentrations, the data show two interesting features. Firstly, the percent increase in F_0 at low Mg ion concentrations with respect to the saturating ones was reduced from an average value of 76% in nonphosphorylated chloroplasts to 44% in phosphorylated chloroplasts. This observation is most simply explained in terms of both LHCP II subpopulations being cation sensitive. The second feature of interest in Fig. 4 is the shift to higher Mg^{2+} concentrations of the maximal F_0 fluorescence value following phosphorylation. This effect can probably be explained in terms of the balance of electrostatic charges required to bring about partial LHCP II energy uncoupling from the PS II reaction centres. Phosphorylation of LHCP II presumably leads to an increased negative-charge density in the LHCP II-PS II matrix domains of the thylakoids, an effect which permits a partial LHCP II energy uncoupling in the presence of a higher concentration of cations.

Fig. 1 shows that at cation concentrations below those at which the maximum F_0 values were

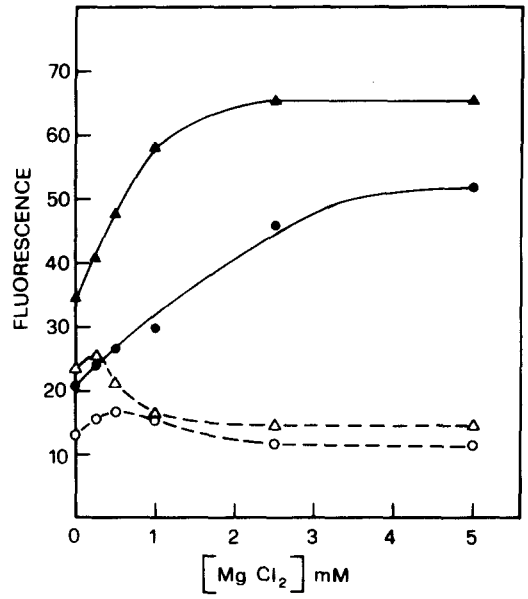

Fig. 4. Titration with $MgCl_2$ of the F_0 and F_M fluorescence induction parameters for phosphorylated and nonphosphorylated spinach thylakoids. Open symbols: F_0; closed symbols: F_M; circles: phosphorylated; triangles: nonphosphorylated. After 10 min in phosphorylating conditions both control and phosphorylated thylakoids were diluted 200 times with ice cold buffer at the desired $MgCl_2$ concentration. Measurement of the fluorescence induction parameters was initiated at least 1 h later.

TABLE II

THE EFFECT OF CATIONS ON THE VELOCITY OF PS II ELECTRON TRANSPORT AND THE F_0 FLUORESCENCE LEVEL

Electron transport units: nmol per μg Chl per h; F_0 fluorescence: relative units.

	$[MgCl_2]$ (mM)			[NaCl] (mM)		
	0	0.25	5	0	25	100
Electron transport	73	86	116	76	96	132
F_0 fluorescence	27	41	30	31	51	37

measured, this parameter declines substantially, as has been previously noted for Mg ions [10]. In terms of a model in which cations influence both energy transfer between the PS II antenna and PS I ('spill-over') and energy transfer from LHCP II to the PS II reaction centres, this fluorescence quenching could be due to an increase in either to these parameters. We have attempted to distinguish between these two possibilities by measuring the steady-state photochemical activity of PS II at low light intensity where the rate of electron transport is almost a linear function of the incident light intensity. In the case of an increased LHCP II-PS II energy coupling we would expect an increased PS II photochemistry, while a 'spill-over' type increase would lead to an opposite result. The data (Table II) show a significant decrease in the photochemical activity of PS II upon lowering either the Na or Mg ion concentration below 25 mM and 0.25 mM, respectively, thus favouring the idea that it is a 'spill-over'-type mechanism which leads to the quenching of F_0 fluorescence at very low cation concentrations. It should be noted that these decreases in photochemical activity (Mg^{2+} : −15%, Na^+ : −21%) are somewhat less than the corresponding F_0 decreases (Mg^{2+} : −34%, Na^+ : −39%). This is expected if the partially uncoupled LHCP II were to transfer energy directly to PS I at very low cation concentrations (below 0.25 mM for Mg^{2+} and 25 mM for Na^+). Such an interpretation is consistent with the observation that the ratio of the F_M values (excitation at 475 nm with respect to 435 nm) decreases only at very low cation concentrations (below 0.5 mM Mg^{2+}, Ref. 10).

Discussion

In the present study it is demonstrated that the increased F_0 fluorescence emission encountered upon lowering the screening cation concentration of the chloroplast suspension medium originates from LHCP II. This is achieved by a detailed comparison of the cation effect on the fluorescence excitation spectrum with that of (a) the chlorina barley mutant lacking LHCP II and (b) thylakoids subjected to LHCP II phosphorylation. As far as we are aware this is the most direct evidence implicating cations in the energy coupling of LHCP II to PS II reaction centres.

In a previous communication from this laboratory [10] we failed to observe a significant difference in the F_M/F_0 ratio when fluorescence was excited at either 475 nm or 435 nm in chloroplasts suspended at a low Mg^{2+} concentration. This evidence was interpreted as indicating no significant LHCP II uncoupling from PS II reaction centres in the concentration range 0.5–2.5 mM. This is in contrast with our present interpretation. We believe that the previous inability to observe this phenomenon was due to the relatively small increase in the F_0 fluorescence encountered upon lowering the cation concentration in the previous experiments.

Studies with thylakoids in which the LHCP II was extensively phosphorylated, show that the F_0 changes as a function of cation concentration were reduced, but by no means eliminated, after the migration of the 'mobile' LHCP II from the LHCP II-PS II matrix to PS I. This suggests that both the 'mobile' and the 'nonmobile' LHCP II subpopulations are cation sensitive.

The mechanism of the energy coupling influence of cations is not known. As with most other cation effects on energy-transfer parameters in thylakoids, the screening of negative electrostatic charge in the sense of the Gouy-Chapman theory seems to be involved. This conclusion is suggested by (a) the observation that monovalent, divalent and trivalent cations were effective in influencing LHCP II energy coupling to PS II reaction centres at the concentrations required to

fit the expectations of that theory and (b) the observation that the F_0 maximum was shifted to higher cation concentrations in thylakoids in which the negative surface charge density of the LHCP II-PS II domains was increased by LHCP II phosphorylation.

References

1 Izawa, S. and Good, N.E. (1966) Plant Physiol. 41, 544–552.
2 Gross, E.L. and Prasher, S.H. (1974) Archiv. Biochem. Biophys. 164, 460–468.
3 Barber, J. and Chow, W.S. (1974) FEBS Lett. 105, 510.
4 Jennings, R.C., Garlaschi, F.M., Gerola, P.D., Etzion-Katz, R. and Forti, G. (1981) Biochim. Biophys. Acta 638, 100–107.
5 Andersson, B. and Anderson, J.M. (1980) Biochim. Biophys. Acta 593, 427–440.
6 Loos, E. (1976) Biochim. Biophys. Acta 446, 314–321.
7 Butler, W.L. (1978) Ann. Rev. Plant Physiol. 29, 345–378.
8 Wong, D. and Govindjee (1981) Photochem. Photobiol. 33, 103–108.
9 Hipkins, M.F. (1978) Biochim. Biophys. Acta 502, 514–523.
10 Jennings, R.C. (1984) Biochim. Biophys. Acta 766, 303–309.
11 Kyle, D.J., Kuang, T.Y., Watson, J.L. and Arntzen, C.J. (1984) Biochim. Biophys. Acta 765, 89–96.
12 Larsson, U.K. and Andersson, B. (1985) Biochim. Biophys. Acta 809, 396–402.
13 Torti, F., Gerola, P.D. and Jennings, R.C. (1984) Biochim. Biophys. Acta 767, 321–325.
14 Mills, J.D. and Barber, J. (1978) Biophys. J. 21, 257–272.
15 Holzwarth, A.R., Wendler, J. and Haehnel. W. (1985) Biochim. Biophys. Acta 807, 155–167.
16 Hodges, M. and Moya, I. (1987) Photosynthesis Res., 13, 125–141.
17 Hodges, M., Briantais, J.-M. and Moya, I. (1987) Biochim. Biophys. Acta 893, 480–489.
18 Ried, A. (1972) in Proceedings of the 2nd International Congress on Photosynthesis Research, Stresa 1971 (Forti, G., Avron, M. and Melandri, A., eds.), Vol. I, pp. 763–772, Dr. W. Junk Publishers, Dordrecht.
19 Horton, P. and Black, M.T. (1981) Biochim. Biophys. Acta 635, 53–62.
20 Telfer, A., Hodges, M., Millner, P.A. and Barber, J. (1984) Biochim. Biophys. Acta 724, 164–175.
21 Jennings, R.C., Garlaschi, F.M. and Gerola, P.D. (1983) Biochim. Biophys. Acta 722, 144–149.
22 Hodges, M. and Barber, J. (1984) Biochim. Biophys. Acta 767, 102–107.
23 Jennings, R.C., Islam, K. and Zucchelli, G. (1986) Biochim. Biophys. Acta 850, 483–489.