Biochimica et Biophysica Acta, 766 (1984) 141–147
Elsevier

BBA 41522

ENERGY TRANSFER AND DISTRIBUTION IN THE RED ALGA *PORPHYRA PERFORATA* STUDIED USING PICOSECOND FLUORESCENCE SPECTROSCOPY

KERRY K. KARUKSTIS and KENNETH SAUER

Department of Chemistry and Laboratory of Chemical Biodynamics, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720 (U.S.A.)

(Received December 21st, 1983)

Key words: Energy transfer; Fluorescence lifetime; Chlorophyll; Photosynthesis; (P. perforata)

The detailed process of excitation transfer among the antenna pigments of the red alga *Porphyra perforata* was investigated by measuring time-resolved fluorescence emission spectra using a single-photon timing system with picosecond resolution. The fluorescence decay kinetics of intact thalli at room temperature revealed wavelength-dependent multi-component chlorophyll *a* fluorescence emission. Our analysis attributes the majority of chlorophyll *a* fluorescence to excitation originating in the antennae of PS II reaction centers and emitted with maximum intensities at 680 and 740 nm. Each of these fluorescence bands was characterized by two kinetic decay components, with lifetimes of 340–380 and 1700–2000 ps and amplitudes varying with wavelength and the photochemical state of the PS II reaction centers. In addition, a small contribution to the long-wavelength fluorescence band is proposed to arise from chlorophyll *a* antennae coupled to PS I. This component displays fast decay kinetics with a lifetime of approx. 150 ps. Desiccation of the thalli dramatically increases the contribution of this fast decay component.

Introduction

Red algae contain phycobilins and chlorophyll *a* as accessory pigments involved in the efficient collection of light and transfer of excitation energy to photochemical reaction centers. The phycobiliprotein pigments are organized into antenna complexes called phycobilisomes which transfer excitation to the chlorophyll *a* antenna of Photosystem II (PS II), while a separate antenna of chlorophyll *a* primarily serves Photosystem I (PS I) [1]. The process of excitation transfer among the antenna pigments of phycobilisome-containing organisms has been monitored using kinetic measurements of the rise and decay of the wavelength-resolved fluorescence emission components [2–4]. The fluorescence emission attributed to chlorophyll pigments in red algae has often been assumed to originate in chlorophyll pools associated only with PS II [2,5–12], but a number of investigations [13–19] have considered the possibility of fluorescence emission from the chlorophyll *a* antenna of both photosystems at room temperature.

The suggestion of fluorescence from PS I chlorophyll in red algae has arisen from the observation in some species of a room-temperature fluorescence emission band at long wavelength, analogous to the low-temperature 735-nm fluorescence emission in green plants attributed to the antenna chlorophylls associated with PS I [1,20]. Murata and Takamiya reported the observation of a long-wavelength room-temperature fluorescence band at 720 nm in the red alga *Porphyra yezoensis* [19]. This band was sensitized by 435 nm light and

Abbreviations: PS I, PS II, Photosystem I, II; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

attributed to chlorophyll *a* belonging to Photosystem I. However, illumination of *P. yezoensis* with phycobilisome-sensitizing 500 nm light produced an emission band at 685 nm and a shoulder at 730–740 nm; both bands were proposed to arise from chlorophyll *a* coupled to Photosystem II [19]. The marine red alga *Porphyra perforata* also exhibits a long-wavelength fluorescence emission band at room temperature that is sensitized by excitation of chlorophyll *a* [14]. Fork et al. [14] reported that when excited by blue light (433 nm), *P. perforata* shows an emission spectrum with a major peak at 732 nm and a minor inflection around 687 nm. Illumination of a photosynthetically active thallus with nonsaturating monochromatic green (550 nm) light at physiological temperatures results in an emission spectrum with an allophycocyanin fluorescence band at 660 nm and chlorophyll fluorescence bands at 690 and 730 nm [14]. The excitation spectra for the 685 and 730 nm fluorescence bands were suggestive that chlorophyll *a* molecules of PS II and of PS I, respectively, were responsible for these emission bands. This interpretation differs from that of Murata and Takamiya [19] with regard to the contribution of PS II chlorophyll *a* fluorescence to the long-wavelength emission band. No heterogeneity in the origin of this chlorophyll fluorescence band was proposed by Fork et al. [14] in contrast with the results of Murata and Takamiya [19].

Furthermore, Fork and Öquist found that desiccation produces a dramatic increase in the relative long-wavelength fluorescence yield, with a maximum shifted to 737 nm and a decrease of fluorescence in the 690 nm region [21]. They proposed that desiccation affects the energy distribution between the two photosystems by increasing the transfer of quanta absorbed by the phycobilins associated with PS II to the chlorophyll *a* of PS I [13,17,21,22]. Such an adaptive mechanism may allow *P. perforata,* an intertidal alga that is periodically exposed to conditions of high light intensity and low water potential, to avoid photodamage. Although this alga loses photosynthetic activity as it undergoes desiccation at low tide [23], it still shows photochemical activity of PS I in the dry state, and rewetting of dry *P. perforata* causes a rapid activation of electron transport and a restoration of in vivo fluorescence emission characteristics [13]. Tolerance to desiccation appears to be a critical physiological feature defining intertidal zonation of *P. perforata.*

We have measured the fluorescence decay kinetics of *P. perforata* at room temperature to investigate the characteristics and origin of the chlorophyll fluorescence emission bands. In particular, we have focused on the question of the number of contributing chlorophyll *a* components (i.e., PS I and/or PS II antenna complexes) to the long-wavelength fluorescence emission. The samples for investigation of the wavelength-dependent fluorescence decay components in *P. perforata* included photosynthetically active thalli in natural seawater, photoinactive thalli from DCMU treatment, and desiccation-tolerant thalli produced from air drying. We conclude that two fluorescence emission bands with maxima at 680 and 740 nm arise from chlorophyll *a* associated with PS II. Furthermore, we find an additional kinetic component in the long-wavelength region that may be attributed to fluorescence from chlorophyll *a* coupled to PS I and centered at 730 nm. Our time-resolved studies provide evidence of chlorophyll fluorescence heterogeneity that is not clearly revealed in steady-state measurements.

Materials and Methods

P. perforata was collected at Bodega Bay, California and generously provided by Professor John West, Department of Botany, University of California, Berkeley. Thalli were kept in natural seawater at 11°C and dark-adapted for at least 1 h prior to use. Fluorescence spectra were measured for thalli in natural seawater at room temperature (24°C). Photoinactive samples were prepared by addition of 10 μM DCMU to dark-adapted thalli in seawater. For desiccation studies, drying was induced by passing an air stream over pieces of the thalli in the presence of cool white light.

The fluorescence excitation source was a Spectra Physics synchronously pumped mode-locked dye laser (SP 171 argon ion laser, SP 362 mode locker, and modified SP 375 dye laser). Thalli (one cell layer thick) were excited with pulses of 8 ps half-maximum full-width duration. The excitation wavelength of 590 nm is efficient at simultaneously exciting chlorophyll fluorescence emitted

at both 690 and 730 nm [14]. As a consequence of absorption band overlap, presumably some of the 690-nm chlorophyll fluorescence results from excitation of the phycobilisomes followed by energy transfer to chlorophyll molecules rather than direct absorption by chlorophyll. The maximum laser pulse intensity (10^7 photons/cm^2) was attenuated with neutral density filters for intensity-dependence experiments. Fluorescence was detected from the same side of the algal thallus as excitation occurred. The single-photon timing system and numerical analysis methods have been described previously [24–27]. Fluorescence decay data were resolved into a sum of exponential decays with a lifetime resolution limit of 50 ps. Plots of the deviations of the measured fluorescence decay from the results of the deconvolutions indicate how well the presumed decay law matches the experimental data. We may further evaluate the goodness of fit by the reduced chi-square (χ^2_R) test, with values near 1.00 indicating that the residuals of the measured curve about the fitted curve are normally distributed and due only to statistical noise [28].

Results

Fig. 1 presents the time-resolved emission spectrum of the fluorescence decay components of photosynthetically active *P. perforata* in seawater at 24°C excited with non-saturating light (less than 10^6 photons/cm^2 per pulse) at 590 nm. The low intensity of the excitation pulses allows Fig. 1 to be considered as a representation of the initial fluorescence state (F_0) in which all photochemical reaction centers are open. Emission peaks for the total fluorescence intensity appear at approx. 660, 680 and 730 nm, in agreement with the steady-state fluorescence emission spectra previously reported [14]. At wavelengths below 690 nm, only two exponential fluorescence decay components can be resolved (e.g., $\chi^2_R = 1.07$ for a two-exponential fit at 660 nm; $\chi^2_R = 1.06$ for the analogous three-exponential fit). The major component, of short lifetime, is centered at 660 nm and the minor component, of somewhat longer lifetime, has a maximum at 680 nm. At longer wavelengths, three exponential fluorescence decay components are resolved (e.g., $\chi^2_R = 2.17$ and 1.35 for two- and three-exponential fits, respectively, at 690 nm; $\chi^2_R = 2.09$ and 1.20 for two- and three-exponential fits, respectively, at 730 nm). The fast decay component shows a clear maximum at approx. 730 nm; however, the two slower decay components have broad bands in the 730–750 nm region. At long wavelengths (at least 700 nm), a rise component is evident, but we are unable accurately to determine its risetime due to limitations in our detection system. We estimate it to be less than 50 ps, in agreement with induction times measured for chlorophyll fluorescence in *Phorphyridium cruentum* [2].

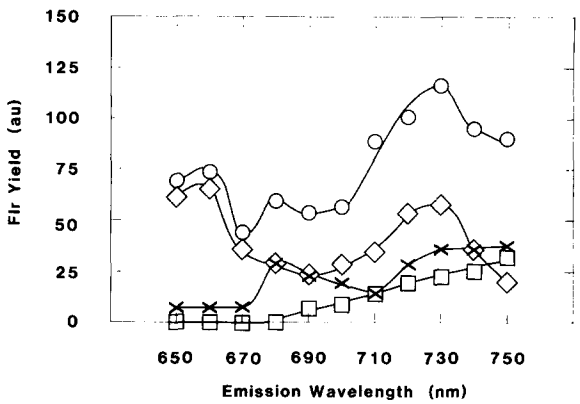

Fig. 1. Total yield and yields of the components of the fluorescence (Flr) decay of *P. perforata* in natural seawater using low excitation intensity at 590 nm (F_0 level) as a function of emission wavelength. (○) Total yield; (□) yield of the slow (1700–2400 ps) component; (x) yield of the middle (300–400 ps) component; and (◇) yield of the fast (125–150 ps) component.

Fig. 2 shows the effect of emission wavelength on the fluorescence lifetimes of the decay components in the F_0 state. The fast phase has a lifetime of approx. 125 ps at the 660 nm band and about 150 ps at the 730 nm band. The middle phase centered at 680 and 730 nm is characterized by average lifetimes of 380 and 340 ps, respectively. The long-wavelength slow decay component has a lifetime averaging 2 ns. The differences in the reported lifetimes of the fast decay component at 660 and 730 nm and of the middle decay component at 680 and 730 nm are within the experimental error of our measurements and not considered significant.

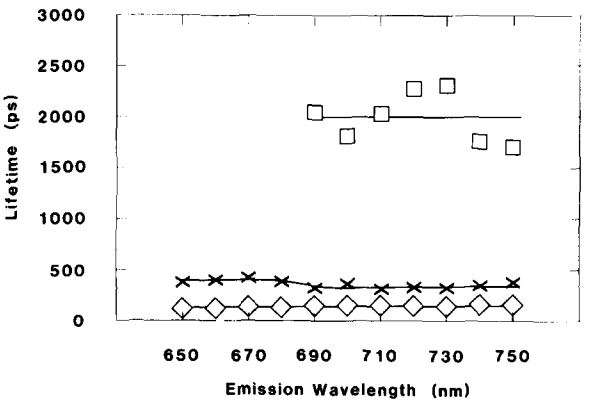

Fig. 2. Lifetimes of the components of the fluorescence decay of *P. perforata* at F_0 as a function of emission wavelength. Experimental conditions and symbols as in Fig. 1.

The emission spectrum of the same sample upon addition of 10 μM DCMU and exposure to high excitation intensity is constructed in Fig. 3. Under these conditions, PS II reaction centers are closed to photochemistry, and maximum fluorescence is attained (F_{max}). The spectrum shows a clear maximum emission at 740 nm with a shoulder at 695 nm and the edge of a small peak at 650 nm. A fast decay component has maximum intensity at 650 nm and in the 720–750 nm region, and a middle decay component is resolved at shorter wavelengths with a broad fluorescence emission band in the 670–700 nm region. The major contribution to the total (and also variable) fluorescence is from

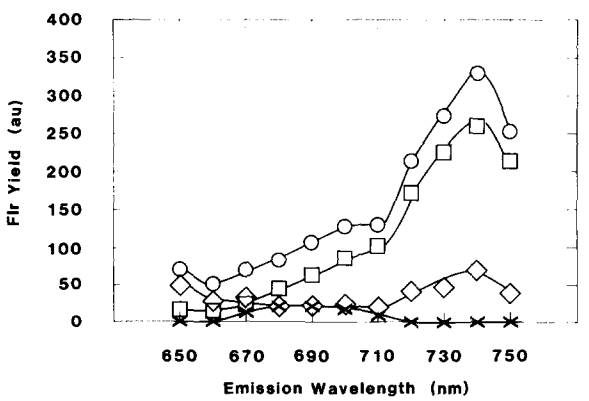

Fig. 3. Total yield and yields of the components of the fluorescence (Flr) decay of P. perforata in natural seawater using high excitation intensity at 590 nm (F_{max} level) as a function of emission wavelength. Thalli were treated with 10 μM DCMU prior to illumination. Symbols are defined as in Fig. 1.

a slow decay component with a peak at 740 nm and a shoulder at 695 nm. As for the F_0 case, we are unable accurately to determine the risetime of a rise component present at wavelengths of at least 700 nm. Typical χ^2_R values for two- and three-exponential fits are 1.56 and 1.14, respectively, at 660 nm, 2.46 and 1.23, respectively, at 690 nm; and 1.10 and 1.09, respectively, at 730 nm, supporting our analysis of three-exponential fits only at shorter wavelengths. Fig. 4 presents the associated lifetimes of the F_{max} fluorescence decay components: a 115 ps component at wavelengths below 690 nm and a 150 ps component at longer wavelengths; a 380 ps component at wavelengths below 690 nm; and a slow decay component of approx. 1700 ps. The differences in the fast decay component lifetime at short (no greater than 690 nm) and long wavelength are within the experimental error of our measurements.

The fluorescence decay kinetics of desiccated (air-dried) *P. perforata* at selected wavelengths is summarized in Table I. The maximum fluorescence appears at 730 nm and is dominated by the contribution of the fast decay component. Only two decay components were resolved at 660 or 690 nm; a small amount of a slow-decaying component is present at 730 nm. No rise component is evident at 730 nm. When thalli are treated with DCMU to close PS II reaction centers prior to desiccation, only a small amount of fast-decaying component is observed at long wavelengths. The fluorescence decay is instead dominated by the slow and middle decay components.

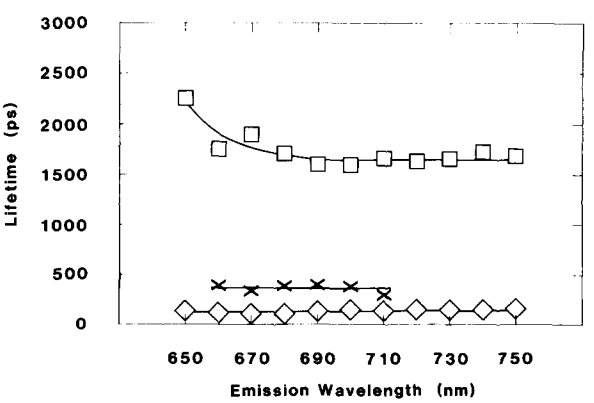

Fig. 4. Lifetimes of the components of the fluorescence decay of *P. perforata* at F_{max} as a function of emission wavelength. Experimental conditions and symbols as in Fig. 3.

Discussion

The fluorescence decay kinetics of *P. perforata* reveals some complexities in the assignment of the room-temperature fluorescence bands. The excitation and emission wavelengths used preclude observation of distinct fluorescence emissions from the individual phycobilin pigments of the phycobilisomes. The principle of systematic energy migration from short-wavelength to long-wavelength chromophores implies that the short-wavelength fluorescence in the 660 nm region arises predominantly from the major allophycocyanin pigment of the phycobilisomes [29], with a characteristic lifetime of 120 ps at F_0 and F_{max}. This value is in excellent agreement with the allophycocyanin fluorescence emission kinetics measured in intact *Porphyridium cruentum* [2], $\tau = 118$ ps at 660 nm. In air-dried *P. perforata,* the increased fluorescence decay time of allophycocyanin, $\tau = 155$ ps, is likely to be associated with the predicted [13,21,22] desiccation-induced alterations in phycobilin energy transfer to PS II and accompanying increase in transfer to PS I.

In the F_0 state, the 680 nm band has been attributed to excitation from chlorophyll *a* coupled to PS II reaction centers [14]. We propose that the middle decay component of the 680 nm band, with a fluorescence lifetime of 380 ps, originates in the chlorophyll *a* antenna of PS II and presumably arises from excitation lost in transit to the reaction center [26]. In DCMU-closed PS II reaction centers, the chlorophyll fluorescence emission at 680 nm has an additional component with a lifetime of 1700 ps. This component is analogous to the slow component of chlorophyll fluorescence in higher plant chloroplasts which has been attributed [26] to fluorescence arising from charge separation in a closed reaction center (P^*-680 I $Q^- \rightarrow P^+$-680 I^- Q^-) followed by radical pair recombination of the oxidized primary electron donor, P^+-680, and the reduced pheophytin primary electron acceptor, I^- [31,32]. Excitation arising from the repopulation of the excited singlet state of chlorophyll (P^+-680 I^- $Q^- \rightarrow P^*$-680 I Q^-) may be transferred from P^*-680 to the antenna chlorophyll molecules and emitted as the slow decay component of fluorescence. The small contribution of a fast decay component in the 680 nm region is presumably due to emission from the minor biliprotein allophycocyanin B [29,33,34], a generally occurring long-wavelength absorbing

TABLE I

FLUORESCENCE DECAY KINETICS OF AIR-DRIED *P. PERFORATA*

Amplitudes (α), lifetimes (τ), and fluorescence yields (ϕ) of the fluorescence decay kinetics in air-dried *P. perforata* with an excitation wavelength of 590 nm and emission wavelengths as stated. Amplitudes are normalized such that $\Sigma\alpha = 1$ at each emission wavelength. The fluorescence yields at each emission wavelength are given as relative yields with respect to ϕ_{total} (= 100) at 730 nm for the air-dried sample.

Emission (λ) (nm)	Air-dried			DCMU-treated prior to air-drying		
	α	τ (ps)	ϕ	α	τ (ps)	ϕ
660	0.03	550	0.5	0.03	785	3.5
	0.97	155	6.0	0.97	175	25.7
			— +			— +
			6.5			29.2
690	0.12	460	8.8	0.21	1700	62.2
	0.88	170	25.3	0.70	620	74.9
			— +			
			34.1	0.09	165	2.4
						— +
						139.5
730	0.01	1635	9.7	0.17	1960	84.0
	0.12	430	22.4	0.73	650	116.7
	0.87	175	67.9	0.10	125	3.1
			— +			— +
			100			203.8

pigment of the phycobilisomes of cyanobacteria and red algae [29,34] with a maximum fluorescence emission at 680 nm [29].

In air-dried thalli, the lifetime of the middle phase at 690 nm is increased to approx. 465 ps, which is suggestive of a less-coupled association of the chlorophyll *a* antenna molecules with the PS II reaction centers [13,21,22]. This alteration would serve to decrease photodynamic damage in strong light by decreasing the probability of PS II reaction center closure from phycobilin-absorbed excitation energy [13,21,22]. The absence of a slow decay component at 690 nm in air-dried samples is also indicative of the prevention of PS II trap closure upon desiccation. However, samples treated with DCMU prior to air-drying retain PS II reaction centers in the closed state upon desiccation.

The variable decay kinetics of the long-wavelength fluorescence region in *P. perforata* is more complex to attribute. As a consequence of its sensitivity to the state of the PS II reaction center, the variable component of the fluorescence decay, the slow component, appears to arise from chlorophyll *a* associated with PS II. On the other hand, the fast phase fluorescence at 730 nm, with a fluorescence intensity insensitive to the state of the PS II reaction center, is proposed to arise from excitation lost in transit to the PS I reaction center from the chlorophyll *a* antennae associated with PS I. This decay component is the major contribution to the fluorescence at 730 nm of air-dried thalli. The absence of the middle decay component at long wavelengths may be a consequence of our inability to resolve small contributions of this component and a fast decay phase in the presence of a large amount of a slower-decaying fluorescence component. Closure of PS II reaction centers with DCMU prior to desiccation prevents the appearance of a large contribution of fast-decaying component at long wavelengths. Presumably, the mechanisms responsible for the enhancement of this component upon air-drying are inoperative with prior PS II closure.

A possible explanation for the heterogeneous origin of the PS II chlorophyll *a* emission at 680 and 730 nm is that there are two types of PS II reaction center-chlorophyll *a* complexes: those associated with phycobilisomes and those completely independent. The PS II units associated with phycobilisomes possess chlorophyll *a* antennae that fluoresce at the shorter wavelength, 680 nm. The independent PS II units, like PS I untis which are not directly connected to the phycobilisomes, exhibit chlorophyll *a* fluorescence at 730 nm. Diner [35] proposed a similar model for wild-type *Cyanidium caldarium* to explain O_2-activation studies in which only one-half of the PS II reaction centers sensitive to chlorophyll excitation were also affected by phycobilin excitation. However, we have no further evidence to support such a proposal of a heterogeneity of PS II complexes in *P. perforata.*

Conclusion

Our investigation of the room-temperature chlorophyll fluorescence decay kinetics of the red alga *P. perforata* reveals distinct PS II chlorophyll emission bands at 680 and 740 nm and a small contribution of PS I chlorophyll fluorescence at approx. 730 nm. These results are analogous to the steady-state fluorescence measurements for another species of the genus, *P. yezoensis.* Although these species of red alga are unusual for their room-temperature long-wavelength fluorescence emission from chlorophyll *a* associated with PS I, most of the long-wavelength emission can be attributed to PS II. Our analysis can be compared to a steady-state fluorescence study of Wang et al. for a low-temperature long-wavelength fluorescence emission band in the red alga *P. cruentum* [18]. They propose that a 718 nm emission band from 550 nm excitation at 77 K arises mostly from PS II chlorophyll emission. This proposal is in contrast to an earlier attribution [1,20] of the 718-nm emission to PS I, as a consequence of excitation transferred from PS II. Our ability to investigate the time-resolved fluorescence decay components of *P. perforata* enables us more conclusively to attribute the origins of the heterogeneous room-temperature chlorophyll *a* fluorescence emission.

Acknowledgements

We thank Professor John West, Department of Botany, University of California, Berkeley for providing the specimens of *P. perforata.* This research was supported by the Director, Office of Energy

Research, Office of Basic Energy Sciences, Division of Biological Energy Conversion and Conservation of the Department of Energy, under contract DE-AC03-76SF00098, and by a grant from the National Science Foundation (PCM82-10524). One of us (K.K.K.) wishes to acknowledge support from a National Institutes of Health National Research Service Award (1F32 GM08617) and a McKnight Foundation grant.

References

1 Ley, A.C. and Butler, W.L. (1976) Proc. Natl. Acad. Sci. USA 73, 3957–3960
2 Porter, G., Tredwell, C.J., Searle, G.F.W. and Barber, J. (1978) Biochim. Biophys. Acta 501, 232–245
3 Brody, S.S., Tredwell, C.J. and Barber, J. (1981) Biophys. J. 34, 439–449
4 Brody, S.S., Porter, G., Tredwell, C.J. and Barber, J. (1981) Photobiochem. Photobiophys. 2, 11–14
5 French, C.S. and Young, V.K. (1952) J. Gen. Physiol. 35, 873–890
6 Brody, S.S. and Rabinowitch, E. (1957) Science 125, 555
7 Tomita, G. and Rabinowitch, E. (1962) Biophys. J. 2, 483–499
8 Singhal, G.S. and Rabinowitch, E. (1969) Biophys. J. 9, 586–591
9 Murata, N. (1969) Biochim. Biophys. Acta 172, 242–251
10 Murata, N. (1970) Biochim. Biophys. Acta 205, 379–389
11 Mar, T., Govindjee, Singhal, G.S. and Merkelo, H. (1972) Biophys. J. 12, 797–808
12 Reid, A. and Reinhardt, B. (1977) Biochim. Biophys. Acta 460, 25–35
13 Fork, D.C. and Hiyama, T. (1972) Carnegie Inst. Yearb. 72, 384–388
14 Fork, D.C., Öquist, G. and Hoch, G.E. (1982) Plant Sci. Lett. 24, 249–254
15 Satoh, K. and Fork, D.C. (1983) Biochim. Biophys. Acta 722, 190–196
16 Satoh, K. and Fork, D.C. (1983) Photosyn. Res. 4, 61–70
17 Satoh, K. and Fork, D.C. (1983) Plant Physiol. 71, 673–676
18 Wang, R.T., Graham, J.-R. and Myers, J. (1980) Biochim. Biophys. Acta 592, 277–284
19 Murata, N. and Takamiya, A. (1967) Plant Cell Physiol. 8, 683–694
20 Butler, W.L. (1978) Annu. Rev. Plant Physiol. 29, 345–378
21 Fork, D.C. and Öquist, G. (1980) Carnegie Inst. Yearb. 80, 39–47
22 Öquist, G. and Fork, D.C. (1979) Carnegie Inst. Yearb. 79, 193–197
23 Wiltens, J., Schreiber, U. and Vidaver, W. (1978) Can. J. Bot. 56, 2787–2794
24 Hartig, P.R., Sauer, K., Lo, C.C. and Leskovar, B. (1976) Rev. Sci. Instrum. 47, 1122–1129
25 Leskovar, B., Lo, C.C., Hartig, P.R. and Sauer, K. (1976) Rev. Sci. Instrum. 47, 1113–1121
26 Haehnel, W., Nairn, J.A., Reisberg, P. and Sauer, K. (1982) Biochim. Biophys. Acta 680, 161–173
27 Turko, B.T., Nairn, J.A. and Sauer, K. (1983) Rev. Sci. Instrum. 54, 118–120
28 Bevington, P.R. (1969) Data Reduction and Error Analysis for the Physical Sciences, p. 89, McGraw-Hill, New York
29 Glazer, A.N. (1983) Annu. Rev. Biochem. 52, 125–157
30 Nairn, J.A., Haehnel, W., Reisberg, P. and Sauer, K. (1982) Biochim. Biophys. Acta 682, 429
31 Klimov, V.V., Allakhverdiev, S.I. and Pashchenko, V.Z. (1978) Dokl. Biophys. (Engl. transl.) 242, 166–169
32 Klimov, V.V., Allakhverdiev, S.I., Demeter, S. and Krasnovskii, A.A. (1979) Dokl.-Biophys. (Engl. transl.) 249, 190–192
33 Glazer, A.N. and Bryant, D.A. (1975) Arch. Microbiol. 104, 15–22
34 Ley, A.C., Butler, W.L., Bryant, D.A. and Glazer, A.N. (1977) Plant Physiol. 59, 974–980
35 Diner, B.A. (1979) Plant Physiol. 63, 30–34