Biochimica et Biophysica Acta, 501 (1978) 208—216

BBA 47441

A DUAL REQUIREMENT FOR PLASTOQUINONE IN CHLOROPLAST ELECTRON TRANSPORT

DAVID A. SADEWASSER and RICHARD A. DILLEY

Department of Biological Sciences, Purdue University, West Lafayette, Ind. 47907 (U.S.A.)

(Received July 27th, 1977)

Summary

When plastoquinone is extracted from lyophilized chloroplasts, electron transport from Photosystem II is inhibited. This inhibition can occur at two levels. One involves the accepted site on the intermediate electron transport chain between the photosystems. The other is associated with the oxidizing side of photosystem II, presumably involving the water oxidation mechanism. Evidence for a dual-site role for plastoquinone is demonstrated by the following findings:

1. Extraction at least 75% of total plastoquinone inhibits all photosynthetic electron transport employing water as the electron source.

2. The photosystem II reaction using iodide as the electron donor and dimethyl-methylene dioxybenzoquinone as a class III acceptor takes place in plastoquinone-extracted chloroplasts. This indicates that class III acceptors intercept electron flow before the plastoquinone pool, and the hypothesized site on the water-splitting side of photosystem II is between the site of water oxidation and iodide donation.

3. Artificial donors, including I^-, do not photoreduce class I acceptors such as methyl viologen in extracted chloroplasts, supporting the previous conclusions that plastoquinone functions between the photosystems.

4. Reconstitution with either plastoquinone A or C in combination with β-carotene restores both the plastoquinone site between the photosystems and water oxidation. β-Tocopherol quinone is also effective in this role.

5. Reconstitution with trimethyl benzoquinone is ineffective in restoring electron transport either from water or artificial donors, indicating a requirement for an isoprenoid or phytol side chain.

Consistent with the work of Okayama (Plant Cell. Physiol. (1974) 15, 95—101), these findings are strong evidence for a dual role for plastoquinone in the

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DMMDBQ, 2,3-dimethyl-5,6-methylene dioxy-*p*-benzoquinone; chl, chlorophyll; Q, quinone.

photosynthetic electron transport scheme. The nature of the requirement implicated in the water oxidation mechanism is still unclear. However, some tentative conclusions based on the effectiveness of plastoquinone analogs in reconstitutions can be made. These lend support to the notion that this function is relatively specific with regard to the structure of the plastoquinone aromatic ring but independent of the length of the isoprene chain beyond one phytol unit.

Introduction

Previous studies on the role of plastoquinone in photosynthetic electron transport have used analytical methods such as extraction and reconstitution [1—4] and light-induced absorbance changes associated with oxidation reduction of the quinone [5—7]. These data have firmly established that plastoquinone functions as an electron carrier in the redox chain between the primary electron acceptor of Photosystem II and cytochrome *f* [1].

Okayama [8] presented evidence for a plastoquinone involvement on the oxidizing side of Photosystem II. His interpretation was based on differential reconstitution of electron flow by plastoquinone using the $H_2O \rightarrow$ dichloroindophenol system when various lipid extraction conditions were used. He demonstrated the photoreduction of dichloroindophenol from diphenylcarbazide after partially extracting plastoquinone and compared these results with the effects of Tris washing, a treatment known to inhibit water oxidation without removing plastoquinone. Reconstitution with plastoquinone restored the water oxidation function only in the partially extracted chloroplasts which were not Tris treated. The results strongly suggest an effect of plastoquinone on the water oxidation mechanism. The possibility cannot be ruled out, however, that the diphenylcarbazide feed to dichloroindophenol does not go through the main chain plastoquinone site while electrons from H_2O do. Dichloroindophenol has previously been shown capable of participating as both an oxidant and reductant in electron transport at multiple sites [9,10]. Diminution of the bulk plastoquinone pool by mild extraction could, therefore, inhibit one system and not the other without the necessity of a direct effect of plastoquinone on the water oxidation mechanism. The weakness of this assay system, then, is the lack of certainty that dichloroindophenol is accepting electrons in a class III mode, i.e. before the main plastoquinone pool.

By using the autooxidizable class III acceptor, 2,3-dimethyl-5,6-methylene dioxy-*p*-benzoquinone (DMMDBQ) [11] and comparing Photosystem II-dependent electron flow using either iodide or water as the electron donor, we can more definitely assess the role of plastoquinone in water oxidation. Primarily from the results with this compound in extracted chloroplasts, evidence is given for a plastoquinone requirement in water oxidation.

This report further demonstrates a degree of generality with regard to plastoquinone analogs and a particular effectiveness of plastoquinone C in water oxidation. Clear deliniation for two separate sites of plastoquinone function is shown in comparison to the conditions necessary to restore electron transport from the artificial donor, iodide, to either methyl viologen or DMMDBQ.

Materials and Methods

Spinach (*Spinacea oleracea*) chloroplasts were isolated as described previously [12]. Final resuspension was in a medium of 0.2 M sucrose, 0.01 M Tricine · NaOH, pH 7.8, 5 mM $MgCl_2$, 1 mM dithioerythritol, with 0.1 M threonine added for cryoprotection [13]. Chlorophyll was determined by the method of Arnon [14].

Chloroplasts were lyophilized for 3 h in a round-bottomed flask with the temperature maintained below 10°C and the lyophilized material was stored at liquid nitrogen temperature.

Extraction and reconstitution procedures have been described previously [15]. Lyophilized chloroplasts were stirred in 30—40 ml dry hexane per mg chlorophyll for 4 h with one change of hexane at 2 h. The addition of 0.02% methanol to the extraction has been found to increase the extraction efficiency of plastoquinone. Efficiency of quinone extraction was analyzed by determining the plastoquinone content of the hexane/methanol extract according to the method of Crane and Dilley [16]. This assay was compared to that obtained by a 1 h acetone extraction. Typically, about 80% of the total plastoquinone was extracted by the hexane. The extracted chloroplasts were dried under reduced pressure. Reconstitutions were conducted by adding quinones to extracted chloroplasts in 5 ml of hexane per mg chl, followed by vacuum removal of all traces of hexane. Reconstitutions were complete in 1.5 h.

Plastoquinone A was obtained from Dr. W.A. Cramer and from Hoffman-LaRoche. Plastoquinone C was obtained from the concentrated hexane extract and purified by thin-layer chromatography [17]. β-Carotene was obtained from Eastman Organic Chemicals. β-Tocopherol quinone was prepared by oxidation from β-tocopherol by a method described elsewhere [18]. α-Tocopherol was purified by thin-layer chromatography from an organic extract of spinach chloroplasts and was kindly supplied by Dr. Rita Barr. Trimethylbenzoquinone was purified by sublimation.

Rates of electron transport were measured from oxygen evolution or consumption on a Clark-type oxygen electrode. Hydroxylamine treatment of freshly isolated chloroplasts is described elsewhere [9].

Dimethyl-methylene dioxybenzoquinone was kindly supplied by Dr. A. Trebst and was dissolved in ethanol/ethylene glycol (1 : 1, v/v).

Results

Plastoquinone involvement in Photosystem II reduction of class III acceptors

In our hexane-extracted chloroplasts we find the expected plastoquinone requirement for electron transfer from Photosystem II to methyl viologen or FeCN using either I^- as the Photosystem II electron donor (line 1, Table I) or water (lines 1 and 2, Table II). It should be noted that rates of electron transport from I^- to methyl viologen in hydroxylamine-treated chloroplasts are significantly less than $H_2O \rightarrow$ methyl viologen rates in freshly isolated or lyophilized chloroplasts. (175 μequiv. · h^{-1} · mg chl^{-1} vs. 302 μequiv. · h^{-1} · mg chl^{-1}). Similarly, $I^- \rightarrow$ DMMDBQ + dibromothymoquinone measures 105 μequiv. · h^{-1} · mg chl^{-1} (Table I, line 3) compared to a rate of 300 for $H_2O \rightarrow$ DMMDBQ

TABLE I

ELECTRON TRANSPORT RATES WITH IODIDE AS THE PHOTOSYSTEM II DONOR

Basic reaction mixtures as in Fig. 1 with concentrations of reactants and inhibitors: 20 mM potassium iodide (I^-), 0.5 mM methyl viologen (MV), 0.5 mM DMMDBQ, 1 μM dibromothymoquinone (DBMIB), 2 μM DCMU. Hydroxylamine treatment was performed on freshly isolated spinach chloroplasts to serve as a control for determining iodide donation rates. The extracted chloroplasts were not NH_2OH treated. The numbers at the top of the table refer to the following treatments: 1, hexane-extracted chloroplasts; 2, reconstituted with β-carotene; 3, reconstituted with β-carotene + plastoquinone at 0.8 mg/mg chlorophyll.

Reaction	Electron transfer rates (μequiv. $\cdot$ $e^- \cdot h^{-1} \cdot$ mg chl^{-1})		
	1	2	3
$I^- \rightarrow$ MV	38	46	121
$I^- \rightarrow$ MV + DCMU	4	4	5
$I^- \rightarrow$ DMMDBQ + DBMIB	62	98	105
$I^- \rightarrow$ DMMDBQ + DCMU	0	1	1

+ dibromothymoquinone in fresh or lyophilized preparations (Table II, line 6). In the I^- donation experiments of Table I there is a significant rate (46 μequiv. $\cdot$ $h^{-1} \cdot$ mg chl^{-1}) in the extracted membranes that is stimulated about 3-fold by plastoquinone A, whereas with water as donor (Table II) there is less activity in the extracted membranes (3 μequiv. $\cdot$ $h^{-1} \cdot$ mg chl^{-1}) with a restoration to 163—183 μequiv. $\cdot$ $h^{-1} \cdot$ mg chl^{-1} with addition of plastoquinone A. We will show below that one reason for less activity in the $H_2O \rightarrow$ methyl viologen case compared to the $I^- \rightarrow$ methyl viologen system is the

TABLE II

ELECTRON TRANSPORT RATES FROM WATER TO ACCEPTOR FOR PHOTOSYSTEM II AND FULL CHAIN REACTIONS IN LYOPHILIZED, EXTRACTED, AND RECONSTITUTED CHLOROPLASTS

The numbers at the top of the table refer to the following treatments: 1, lyophilized chloroplasts; 2, hexane-extracted chloroplasts; 3, reconstituted with β-carotene; 4, reconstituted with β-carotene + plastoquinone A at 1 mg/mg CHL; 5, reconstituted with β-carotene + plastoquinone A at 2 mg/mg CHL; 6, reconstituted with β-carotene + plastoquinone C at 0.5 mg/mg CHL. Basic reaction mixture as in Fig. 1 with electron acceptors and inhibitors as follows: 0.5 mM potassium ferricyanide (FeCN) except in DAD_{ox} reation at 1.5 mM), 0.5 mM methyl viologen (MV), 0.5 mM dimethylbenzoquinone (DMQ), 0.5 mM diaminodurene (DAD), 1 μM dibromothymoquinone (DBMIB) when used to inhibit Photosystem I; 20 μM as an electron acceptor, 0.5 mM dimethyl methylene-dioxy benzoquinone (DMMDBQ), 2 μM dichlorophenyl dimethylurea (DCMU).

Reaction	Electron transfer rates (equiv. $\cdot$ $e^- \cdot h^{-1} \cdot$ mg CHL^{-1})					
	1	2	3	4	5	6
1. $H_2O \rightarrow$ FeCn	224	3	5	14	163	160
2. $H_2O \rightarrow$ MV	302	3	3	22	183	190
3. $H_2O \rightarrow$ DMQ + FeCN + DBMIB	288	0	1	10	78	85
4. $H_2O \rightarrow DAD_{ox}$ + FeCN + DBMIB	437	4	5	12	204	221
5. $H_2O \rightarrow$ DBMIB	127	0	1	6	47	50
6. $H_2O \rightarrow$ DMMDBQ + FeCN + DBMIB	300	0	0	6	186	174
7. $H_2O \rightarrow$ FeCN + DCMU	10	0	0	0	1	0
8. $H_2O \rightarrow$ DMQ + FeCN + DCMU	12	1	1	2	2	0

necessity for plastoquinone in electron flow from H_2O to Photosystem II, and that the amount of plastoquinone required in this activity is greater than that required for electron flow in the main chain. This can best be seen by comparing H_2O and I^- donor rates to a class III acceptor against the rates using methyl viologen (a class I acceptor).

Table I shows that the autooxidizable class III acceptor, DMMDBQ, is readily reduced by I^-, in a DCMU-sensitive Pnotosystem II reaction, without plastoquinone being added back. In agreement with Okayama and Butler [20], we find that adding back β-carotene stimulates Photosystem II activity (line 3, columns 1 and 2, Table I). Fig. 1 shows the concentration effects for β-carotene in the $I^- \rightarrow$ DMMDBQ reaction and the insensitivity of this reaction to plastoquinone A or plastoquinone C addition. It is apparent that reduction of this class III acceptor by Photosystem II does not use the main pool of plastoquinone.

Another Photosystem II donor, diphenylcarbazide, functions with faster rates than I^- in extracted membranes, giving a rate of 172 μequiv. $\cdot e^- \cdot h^{-1} \cdot$ mg chl^{-1}. The control extracted membranes had a zero rate for $H_2O \rightarrow$ FeCN and a rate of 8 μequiv. $\cdot e^- \cdot h^{-1} \cdot$ mg chl^{-1} for the $H_2O \rightarrow$ methyl viologen reaction.

A requirement for plastoquinone on the water oxidizing side of Photosystem II is seen when we measure the $H_2O \rightarrow$ Photosystem II $\rightarrow$ DMMDBQ reaction. Table II, line 6 shows zero rate in the extracted membranes, down from 300 μequiv. $\cdot h^{-1} \cdot$ mg chl^{-1} in the lyophilized membranes, with no stimulation by β-carotene alone (column 3), and a restoration to near 180 μequiv. $\cdot h^{-1} \cdot$ mg chl^{-1} with addition of plastoquinone A or plastoquinone C (columns 5 and 6). Other class II acceptors, dimethylbenzoquinone and diaminodurene (lines

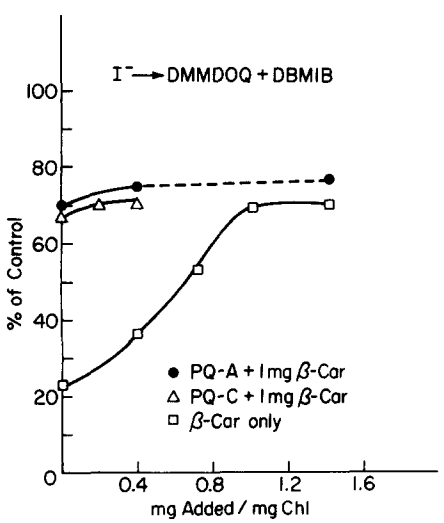

Fig. 1. Restoration of iodide to DMMDBQ + dibromothymoquinone (DBMIB) electron transport with varying concentrations of β-carotene and plastoquinone. Reaction mixture (2 ml) contains 20 mM Tricine · NaOH, pH 8.0, 100 mM KCl, 5 mM $MgCl_2$, 20 mM KI, 0.5 mM DMMDBQ, 2 μM dibromothymoquinone and 30 μg/ml, chlorophyll. Reconstitution was carried out in dry hexane for 1 h at 25°C.

3 and 4) show a similar restoration in reduction rate by plastoquinone A and C, but in those cases we have not compared the water donation to the I^- donation. We assume that DMMDBQ, dimethylbenzoquinone and diaminodurene are all reduced at the same loci, i.e. independent of the inter system plastoquinone pool.

The plastoquinone A restoration on the water side of Photosystem II appears to need more quinone than restoration of the plastoquinone function between the two photosystems. Comparing Fig. 2 ($H_2O \rightarrow$ methyl viologen reaction) and Fig. 3 ($I^- \rightarrow$ methyl viologen reaction), it is clear that 60% restoration of $H_2O \rightarrow$ methyl viologen requires about 2 mg plastoquinone A/mg chl while the $I^- \rightarrow$ methyl viologen reaction is restored to that percent of control by 0.2 mg plastoquinone A/mg chl.

Comparison of plastoquinone A and C and quinone analogs

Plastoquinone C restores the water oxidation function and electron flow between the photosystems more efficiently than plastoquinone A, generally being effective at about one-tenth the amount of plastoquinone A required for maximum restoration (see Figs. 1—3).

The specificity of the plastoquinone requirement was tested by reconstituting the extracted chloroplasts with tocopherols, ubiquinone and other plastoquinones which vary in the length or chemical nature of the isoprenoid chain (Table III). As mentioned before, plastoquinone C, which contains one or two OH^- groups on the isoprene chain, acts identically as plastoquinone A except that about 10-fold less is required. β-Tocopherol quinone restores both plasto-

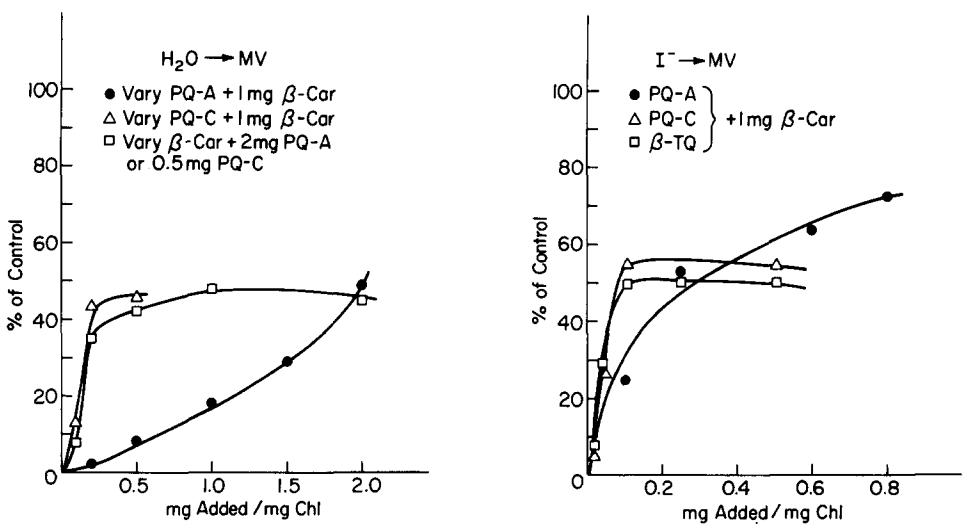

Fig. 2. Effect of varying concentrations of plastoquinones on restoration of water to methyl viologen electron transport in hexane-extracted chloroplasts. Reaction mixture as in Fig. 1 with 0.5 mM methyl viologen and 20 μg/ml chlorophyll. Reconstitution conditions as in Fig. 1.

Fig. 3. Restoration of iodide to methyl viologen electron transport with varying concentrations of plastoquinones. Reaction mixture as in Fig. 1 with 2- mM KI, 0.5 mM methyl viologen, and 30 μg/ml chlorophyll. Reconstitution conditions as in Fig. 1.

TABLE III

RELATIVE RESTORATION OF ELECTRON TRANSPORT BY VARIOUS RECONSTITUTIONS
Ubiquinone-10, α-tocopherol, α- and β-tocopherol quinones and trimethyl benzoquinone were added at 1 mg/mg chlorophyll. All reconstitutions contained β-carotene at 1 mg/mg chlorophyll. See text and Table II for reaction conditions.

Conditions	Percent restoration †			
	$H_2O \rightarrow MV$	$H_2O \rightarrow$ DMMDBQ	$I^- \rightarrow MV$ *	$I^- \rightarrow$ DMMDBQ *
Extracted	1	0	22	41
+ β-carotene	1	0	26	65
+ plastoquinone A **	61	62	—	—
+ plastoquinone C ***	63	58	—	—
+ plastoquinone A + C	59	59	—	—
+ β-tocopherol	48	39	—	—
+ ubiquinone-10	52	47	20	20
+ α-tocopherol	2	1	21	58
+ trimethylbenzoquinone	0	0	18	41
+ α-tocopherolquinone	0	0	17	43

* Versus rates in NH_2OH-treated chloroplasts.
** PQA at 2 mg/mg chlorophyll.
*** PQ-C at 0.2 mg/mg chlorophyll.
† Lyophilized chloroplasts used as a control (see Table II).

quinone sites to about 80% of that with plastoquinone. Plastoquinone A and plastoquinone C together show no additivity of restoration, indicating that one or the other compound probably saturates the sites (Table III). However, plastoquinone A added with β-tocopherol quinone slightly enhances the reconstitution of β-tocopherol quinone. α-Tocopherol, α-tocopherol quinone, and ubiquinone-10 were totally ineffective in restoring either water oxidation or the intermediate chain site. No data are shown for the iodide donation reactions in reconstitutions which restore water oxidation because iodide donation cannot be measured when it is superceded by the physiological water donor to Photosystem II.

One plastoquinone species which did not restore electron transport is trimethylbenzoquinone. Indeed, the presence of this compound added to non-extracted lyophilized chloroplasts in a short heptane "reconstitution" inhibits class III and class I reactions.

Discussion

It is clear from these data that plastoquinone functions both in the redox chain between the photosystems and on the oxidizing side of Photosystem II. The distinction between the two sites is best seen by noting that plastoquinone is required to restore $I^- \rightarrow$ methyl viologen electron transport in hexane-extracted membranes but it is not required for the $I^- \rightarrow$ DMMDBQ reaction (Table I). The latter reaction, using the autooxidizable class III acceptor, is DCMU sensitive and DBMIB insensitive and it occurs in NH_2OH-treated membranes that have no H_2O oxidation activity; hence it is a typical Photosystem II photoreaction. Using H_2O as a donor with DMMDBQ as the acceptor, there is no activity

until plastoquinone is added back (Table II), clearly consistent with the concept that in some way it restores electron flow between H_2O and the Photosystem II reaction center complex. Plastoquinone is not required for the Photosystem II oxidation of donors such as diphenylcarbazide, which release H^+ upon oxidation, at least not in lyophilized, extracted membranes. However, it should be noted that lyophilized chloroplasts suffer apparently an irreparable damage to the proton-accumulating capacity, because we could not obtain the expected H^+ uptake in lyophilized membranes. With this reservation, then, we suggest that plastoquinone is not needed for the processing of protons liberated in Photosystem II oxidations.

Another indication of two sites for plastoquinone function is that lesser amounts of plastoquinone are needed to restore the redox step between the photosystems than to restore H_2O oxidation (Figs. 2 and 3).

As previously shown by Henninger and Crane [26], in $H_2O \rightarrow$ dichloroindophenol electron transport, we find that plastoquinone C is much more effective than plastoquinone A in restoring function either in the redox chain between the photosystems, ($I^- \rightarrow$ methyl viologen, Fig. 3) or in restoring water oxidation (Fig. 2). β-Tocopherol quinone was nearly as effective as plastoquinone A in restoring function at both loci, while α-tocopherol quinone was ineffective at either loci. This suggests that neither the length or degree of saturation of the side chain (the tocopherol quinones have a fully saturated phytol chain) is critical down to 20 carbons. The ring substituents are more critical, a free position on the quinone ring (the plastoquinones, β- or γ-tocopherol quinone) allows activity and α-tocopherol, being fully substituted on the quinone ring, is inactive.

This work supports and extends the work of Okayama [8], who first reported a plastoquinone function on the oxidizing side of Photosystem II, and Warden et al. [21] who showed a transient, free radical ESR signal in chloroplasts having semi-quinone characteristics and seemingly involved on the oxidizing side of Photosystem II.

The nature of plastoquinone involvement in water oxidation is not clear. The redox potential of the plastoquinone/reduced plastoquinone couple near +100 mV [22,23] is not consistent with this couple having a redox function. There are, however, possibilities for plastoquinone redox conversions involving unusual oxidation-reduction states. Wood and Bendall [24] indicate that the couple QH_2^+/QH_2 would have an E_0' value near + 920 mV, and together with the realization that midpoint potentials are sensitive to the local environment [25] it is possible that plastoquinone has an oxidation-reduction function on the oxidizing side of Photosystem II.

Acknowledgements

This work was partially supported by N.I.H. Grant 5R01GM19595, N.S.F. Grant GM-30998 and N.I.H. Career Development Award to R.A.D.

References

1 Amesz, J. (1972) Biochim. Biophys. Acta 301, 35—51
2 Henninger, M.D. and Crane, F.L. (1966) J. Biol. Chem. 241, 5190—5196

3 Krogmann, D.W. and Oliverio, E. (1962) J. Biol. Chem. 237, 3292—3295
4 Wood, P.M. and Crane, F.L. (1965) Biochim. Biophys. Res. Commun. 20, 274—278
5 Amesz, J., Visser, W.M., Van Den Engh, G.J. and Dirks, M.D. (1971) Biochim. Biophys. Acta 256, 370—380
6 Rumberg, B., Schmidt-Mende, P., Wiekard, J. and Witt, H.J. (1963) in Photosynthetic Mechanisms in Green Plants, p. 18, Publ. Natl. Res. Council, Washington 1145
7 Stiehl, H.H. and Witt, H.T. (1968) Z. Naturforsch. 236, 220—224
8 Okayama, S. (1974) Plant Cell. Physiol. 15, 95—101
9 Kok, B., Malkin, S., Owens, O. and Forbush, B. (1966) Energy Conversion by the Photosynthetic Apparatus, Brookhaven Symposia on Biology, Vol. 19, pp. 446—459
10 Neumann, J., Arntzen, C.J. and Dilley, R.A. (1971) FEBS Lett. 16, 164—166
11 Trebst, A., Reimer, S. and Dallacker, F. (1976) Plant Sci. Lett. 6, 21—24
12 Giaquinta, R.T., Dilley, R.A. and Anderson, B.J. (1971) Biochem. Biophys. Res. Commun. 52, 1410—1417
13 Heber, U., Tyankova, L. and Santarius, K.A. (1971) Biochim. Biophys. Acta 241, 578—592
14 Arnon, D.I. (1949) J. Plant Physiol. 24, 1
15 Cox, R.P. and Bendall, D.S. (1974) Biochim. Biophys. Acta 347, 49—59
16 Crane, F.L. and Dilley, R.A. (1963) in Methods of Biochemical Analysis (Glick, D., ed.), Vol. XI, pp. 289—305, Interscience Publishers, New York
17 Dilley, R.A. (1964) Anal. Biochem. 7, 240—246
18 Dilley, R.A. and Crane, F.L. (1963) Anal. Biochim. 5, 531—541
19 Ort, D.R. and Izawa, S. (1973) J. Plant Physiol. 52, 595—600
20 Okayama, S. and Butler, W.L. (1971) J. Plant Physiol. 49, 769—774
21 Warden, J.T., Blankenship, R.E. and Sauer, K. (1976) Biochim. Biophys. Acta 423, 362—478
22 Carrier, J.M. (1967) in Biochemistry of Chloroplasts (Goodwin, T.W., ed.), Vol. 2, pp. 551—557, Academic Press, London
23 Okayama, S. (1976) Biochim. Biophys. Acta 440, 331—336
24 Wood, P.M. and Bendall, D.S. (1976) Eur. J. Biochem. 61, 337—344
25 Dutton, P.L. and Wilson, D.F. (1974) Biochim. Biophys. Acta 346, 165—212
26 Henninger, M.D. and Crane, F.L. (1966) J. Biol. Chem. 242, 1155—1159