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COBALT IONS INHIBIT ELECTRON-TRANSPORT ACTIVITY OF PHOTOSYSTEM II WITHOUT AFFECTING PHOTOSYSTEM I

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Investigations on photosynthesis have greatly benefited by the use of specific inhibitors that affect a specific site of inhibition on the electron-transport chain. We show here for the first time that cobalt (Co^{2+}) ions can be used specifically to inactivate electron donation to the reaction centre of Photosystem (PS) II without affecting PS I reactions. This conclusion is based on the following observations: (1) addition of exogenous electron donors such as NH_2OH does not relieve Co^{2+}-induced inactivation of photoelectron transport or the lowering of steady-state chlorophyll a fluorescence yield; this suggests that the inhibition is beyond the NH_2OH donation site and before the fluorescence quencher Q, i.e., on the reaction centre complex itself. (2) Washing of Co^{2+}-pretreated chloroplasts with isolation buffer to remove Co^{2+} does not relieve Co^{2+}-induced inhibition of Hill activity, suggesting that the Co^{2+} effect is irreversible. (3) Co^{2+} did not alter the PS I reactions. Thus, Co^{2+}-treated chloroplasts can be used to study PS I functions free from PS II reactions in isolated chloroplasts.

Introduction

The electron donor on the oxidizing side of PS II is sensitive to metal ions [1–3]. The heavy metal ions Pb^{2+}, Hg^{2+}, Cd^{2+} and Cu^{2+} affect electron carriers of PS II [1–7]. However, only limited work has been carried out on the effect of these metal ions which are essential as micronutrients and which also affect plant growth and development when they are present in the environment at an elevated level [8,9]. Co^{2+} is an essential micronutrient and is required as a prosthetic group of

several enzymes [10,11]. Excess Co^{2+} reduces the dry matter production and chlorophyll and protein contents in mung beans [12]. Cobalt has been claimed to restore the oxygenase but not the carboxylase function of ribulosebisphosphate carboxylase deactivated by the removal of Mg^{2+} [13].

The present work aims at determining the effect of Co^{2+} on the photoelectron-transport ability of isolated chloroplasts. The results indicate that Co^{2+} inactivates the PS II function, and that this inactivation appears to be due to irreversible inactivation of the PS II reaction centre complex, since the electon donor NH_2OH, which donates to this reaction centre via an endogenous donor, is unable to restore electron flow and Chl a fluorescence yield remains low. furthermore, this effect is specific to PS II because PS I-mediated electron flow remains unaffected by Co^{2+}.

Materials and Methods

Barley (*Hordeum vulgare* L CV 1 B 65) seeds were germinated in petri plates fitted with water-soaked filter papers at 25°C, under continuous illumination (2500 lx), as described earlier [8]. Fully expanded primary leaves from 11-day-old seedlings were used for chloroplast preparation. For some experiments with Chl *a* fluorescence, spinach beets (a local variety of *Beta vulgaris*) were purchased from the local market and fresh leaves were used for chloroplast isolation. Chloroplasts were isolated by homogenizing 5 g leaves in 75 ml ice-cold medium containing 400 mM sucrose, 10 mM Hepes-NaOH buffer (pH 7.6) as described earlier [8].

Chloroplast assays

PS II-supported DCIP photoreduction was measured according to standard procedures [8]. The 3 ml reaction mixture consisted of 50 mM Hepes-NaOH buffer (pH 7.0), 2 mM $MgCl_2$, 10 mM NaCl and 30 μM DCIP. Chloroplasts at a Chl concentration of 15 μg/ml were added to the above reaction mixture and the sample was illuminated for 30 s with saturating (approx. $7.0 \cdot 10^4$ erg $\cdot$ cm^{-2} $\cdot$ s^{-1}) heat-filtered white light. DCIP-supported O_2 evolution was measured in a Yellow Springs Instrument (Yellow Springs, OH) O_2 probe as described earlier [8]. The assay of whole-chain electron transport from H_2O to methyl viologen or PS I-mediated $DCIPH_2$ to methyl viologen photoreduction and associated O_2 uptake were monitored as described earlier [8]. Other details are given in the legends to the figures and tables.

Chl *a* fluorescence emission spectra and yield were measured in a laboratory-assembled spectrofluorometer as described earlier [15]. The details of measurement for Chl *a* fluorescence intensity have been described elsewhere [14,15]. Low-temperature emission spectra and delayed light emission were measured in an Aminco-Bowman spectrofluorometer [15]. Other details are given in the figure legends.

$CoSO_4$ and $CoCl_2$ were purchased from British Drug House, Ltd., and all other chemicals from Sigma Chemical Co., St. Louis. Chloroplasts were incubated with $CoSO_4$ or $CoCl_2$ in the dark at 0–4°C. For control, chloroplasts without Co^{2+}

treatment were also kept for an equal length of time for comparison.

Results and Discussion

The whole-chain electron transport

Fig. 1 shows the effect of Co^{2+} treatment on the whole-chain electron transport as measured by photoreduction of methyl viologen with H_2O as electron donor. Methyl viologen photoreduction and its auto-oxidation which results in O_2 uptake by isolated barley chloroplasts was inhibited by 50% with 3 mM $CoSO_4$ after approx. 12 min incubation and the extent of inhibition increased up to 85% with 8 mM $CoSO_4$. There was a rapid initial decline in the rate of O_2 uptake within 5 min incubation of chloroplasts with the Co^{2+} salt,
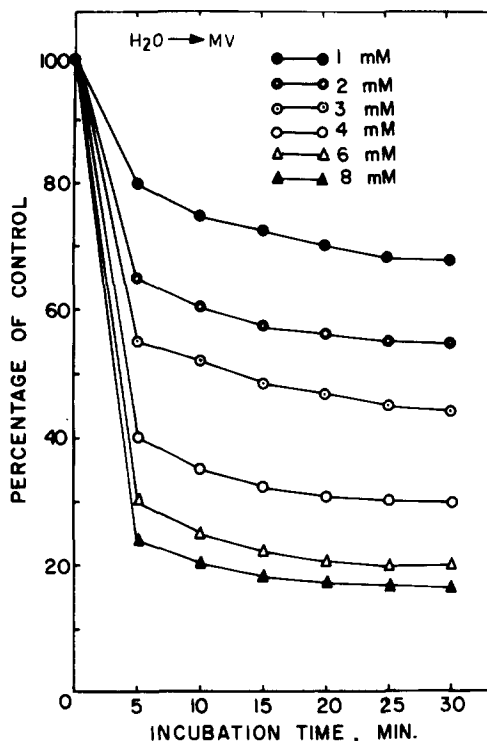

Fig. 1. Time course of inhibition of whole-chain electron transport ($H_2O \rightarrow$ methyl viologen (MV)) with various concentrations of Co^{2+}-treated barley chloroplasts. Control rate without Co^{2+} treatment represents 100% activity = 60 μmol/mg Chl per h. Loss in control chloroplast activity incubated for 30 min in the dark at 4°C was only approx. 3–5% of the initial 100% value.
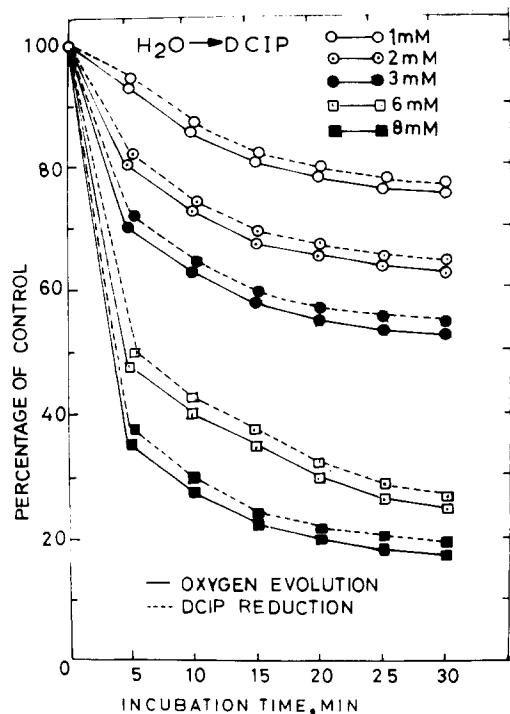
Fig. 2. Time course of inhibition, PS II-catalyzed H_2O to DCIP photoreduction and O_2 evolution with various concentration of Co^{2+}-treated barley chloroplasts. Co^{2+} was used as $CoSO_4$ O_2-evolution control (solid line); 100% activity = 45–50 μmol/mg Chl per h and DCIP reduction (dashed line); 100% activity of control (untreated) chloroplasts = 80–85 μmol/mg Chl per h.

Fig. 3. Effect of various concentrations of $CoSO_4$ on the whole-chain (H_2O to methyl viologen (MV)) electron transport, through PS II (H_2O to DCIP) only, and through PS I ($DCIPH_2$ to methyl viologen) only. Control rate (100%) for H_2O to methyl viologen = 60 μmol/mg Chl per h; for H_2O to DCIP = 50 μmol O_2 evolved/mg/Chl per h, and for $DCIPH_2$ to methyl viologen = 400 μmol O_2 uptake/mg Chl per h. Chloroplasts were treated with $CoSO_4$ for 10 min in the dark at 0–4°C before assay.

and the extent of inhibition increased slightly and gradually with time of incubation (Fig. 1). No differential effect was observed whether chloroplasts were incubated with $CoSO_4$ in light or in darkness (data not shown). Similar results were obtained with $CoCl_2$ showing that the active species is Co^{2+}.

PS II-supported electron transport

Electron flow from H_2O to DCIP is essentially catalysed by PS II [16]. Fig. 2 shows the time-dependent inhibition of DCIP-supported PS II-mediated O_2 evolution by barley chloroplasts with varying concentrations of $CoSO_4$. There was a rapid inhibition of O_2 evolution within 5 min of incubation with Co^{2+}, the extent of which increased with both time of incubation and concentration of $CoSO_4$. Fig. 2 also shows that the extent and development of inhibition were almost the same
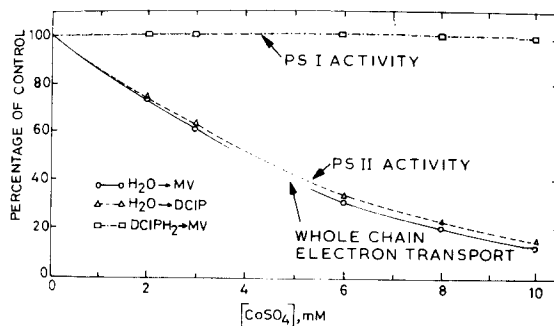
whether O_2 evolution (solid line) or DCIP photoreduction (dashed line) was monitored.

PS I-catalyzed electron transport

Electron flow from the reduced donor $DCIPH_2$ to methyl viologen in the presence of DCMU (1 μM), NaN_3 (3 mM) and methyl viologen (0.1 mM) is catalysed by PS I [17]. Fig. 3 shows that the PS I-catalyzed methyl viologen photoreduction and its subsequent auto-oxidation resulting in O_2 uptake was not affected by Co^{2+} to any significant extent, even with the high concentration (10 mM) of $CoSO_4$ used, while PS II-dependent electron flow decreased progressively with increasing concentration of $CoSO_4$. Similarly, Co^{2+} did not affect PS I electron flow supported by reduced TMPD (data not shown). Thus, Co^{2+}, even at a high concentration, selectively affects PS II activity without affecting that of PS I.

Effect of Co^{2+} on exogenous donor supported PS II-catalysed DCIP photoreduction

It is clear from the above results that Co^{2+} treatment of chloroplasts inhibits PS II-catalyzed electron transport. In order to ascertain whether the Co^{2+}-induced inhibition is due to inactivation of the O_2-evolving system of PS II or due to a block in the intersystem electron-transport chain, the electron transport from exogenous electron

donors, which provide electrons through PS II [18], to DCIP was monitored. Additions of exogenous donors such as $MnCl_2$ (0.3 mM), benzidine (0.5 mM) and NH_2OH (5 mM) to chloroplasts pretreated with various concentrations (1-6 mM) of $CoCl_2$ reduced the extent of Co^{2+}-induced inhibition maximally by 5-8%; thus, the extent of inhibition remained unaltered with and without these exogenous donors. (As diphenylcarbazide produced a red colouration with Co^{2+}, it could not be used as a donor.) Although other electron donors to PS II have not been tested, the inability of NH_2OH to alleviate Co^{2+} inhibition suggests that Co^{2+} affects PS II primary photochemistry. Thus, the extent of inhibition remained unaltered with or without these exogenous donors.

Effect of Co^{2+} on Chl a fluorescence yield and emission spectrum of chloroplasts
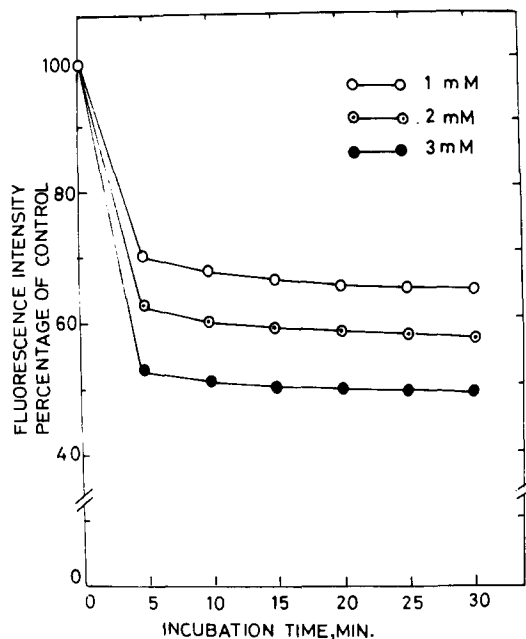
At rate-saturating intensity, the steady-state

level of Chl a fluorescence (F_s) of isolated chloroplasts in the absence of any exogenous acceptor reflects the accumulation of reduced Q, the quencher of Chl a fluorescence and the stable primary acceptor of PS II [19,20,24]. Thus, fluorescence level F_s is a measure of the electron-transport ability of PS II [20,24]. Fig. 4 shows that incubation of chloroplasts with Co^{2+} lowers the room-temperature steady-state (F_s) level of Chl a fluorescence, measured at 685 nm (F_{685}). Cobalt treatment of chloroplasts induced rapid loss in Chl a fluorescence yield within 5 min incubation; the extent of quenching of Chl a fluorescence intensity increased with increase in cobalt concentration. $CoSO_4$ (3 mM) lowered the steady-state fluorescence intensity by 50% of the initial untreated level. We further investigated whether exogenous electron donors such as $MnCl_2$ (0.3 mM) and NH_2OH (5 mM) would restore the Co^{2+} induced lowering of Chl a fluorescence, and observed that none of the above-mentioned electron donors could restore Chl a fluorescence intensity lowered by Co^{2+}, by treating the chloroplasts with 3 mM $CoCl_2$ for 10 min (data not shown). These results indicates that Co^{2+} inactivates photochemical activity of PS II.

Fluorescence spectra provide information on the composition of the photosystems. Cobalt treatment also changes the emission characteristics of
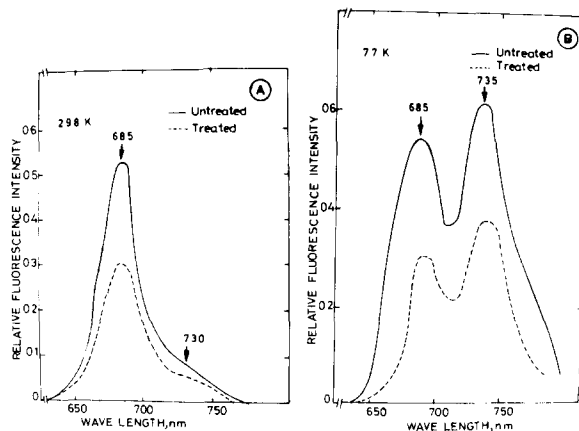

Fig. 4. Time course of inhibition of steady-state Chl a fluorescence intensity (F_s), measured at room temperature, in Co^{2+}-treated chloroplasts. Spinach beet chloroplasts were used. Fluorescence was measured at 685 nm. Control chloroplasts were kept in the dark for the same length of time as treated samples for comparison. Fluorescence intensities were recorded in relative units and percentage quenching was calculated with respect to an untreated control. [Chl] = 4 μg/ml. The existing light intensity was approx. $1 \cdot 10^4$ erg$\cdot$cm$^{-2}\cdot$s^{-1}.

Fig. 5. Effect of Co^{2+}-treatment on (A) room-temperature Chl a fluorescence emission and (B) 77 K Chl a fluorescence emission spectra of isolated chloroplasts. Spinach beet chloroplasts were treated with 2 mM $CoSO_4$ for 10 min before measurements. Low-temperature measurements were recorded in a very dilute sample with narrow slit widths.

the Chl *a* fluorescence of chloroplasts. However, cobalt addition caused no obvious change in the absorption characteristics of chloroplasts (data not shown) and Co^{2+} quenched the 685 nm band (F-685) of the room-temperature emission spectrum by 40%, but the ratio F-685/F-730 did not change much (5.6 to 5.4) on addition of 2 mM $CoSO_4$ (Fig. 5A). Unlike the room-temperature emission spectrum, the 77 K spectrum exhibits peaks at 685 nm (F-685) and at 735 nm (F-735) and a shoulder at 695 nm (F-695) [19–21]. The F-735 band emanates from PS I, which is only weakly fluorescent at room temperature [20,22]. Fig. 5B shows the 77 K Chl *a* emission spectra of Co^{2+}-treated and control chloroplasts. Both F-685 and F-735 bands of Chl *a* emission were suppressed by the presence of $CoSO_4$; however, F-685 was slightly more quenched than the F-735 band. The ratio F-685/F-735 changed from 0.88 in untreated chloroplasts to 0.80 in Co^{2+}-treated chloroplasts. This decrease in Chl *a* fluorescence may partly be due to non-specific quenching of Chl *a* fluorescence by paramagnetic Co^{2+}. However, the absence of NH_2OH-induced enhancement of Chl *a* fluorescence appears to be due to a loss of Q-linked changes of fluorescence yield as NH_2OH donates electrons very close to the PS II reaction centre [20,21].

The delayed light emission spectra measured in chloroplasts treated with and without Co^{2+} also showed a quenching of delayed luminescence emission intensity by 43% of the control by Co^{2+}. As millisecond delayed light emission originates mostly from PS II and reflects chloroplast electron-transport ability [23], and thus the quenching of delayed light emission by Co^{2+} suggests that these affect PS II-mediated chloroplast function [23,25,26].

Effect of washing of Co^{2+}-treated chloroplasts

In order to ascertain whether Co^{2+}-induced inhibition of chloroplast electron-transport activity could be removed by washing the chloroplasts free of Co^{2+}, chloroplast suspensions were incubated with $CoSO_4$ (2 mM) for 15 min and then washed with Co^{2+}-free buffer two or three times. Control chloroplasts were also similarly washed, and DCIP photoreduction was measured in both. Fig. 6 shows that washing of chloroplasts only marginally
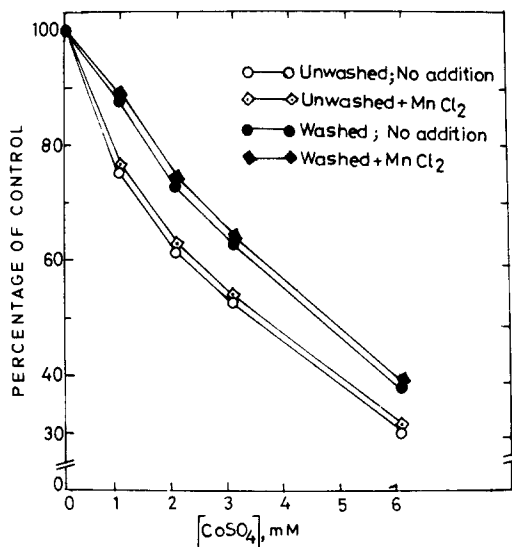

Fig. 6. Effect of washing of Co^{2+}-treated chloroplasts with isolation buffer on DCIP-supported O_2 evolution. Chloroplasts were treated with 4 mM $CoSO_4$ for 15 min in the dark at 0°C before washing. Control chloroplasts were also washed in isolation buffer. Control activity, 100% = 80 μmol/mg Chl per h.

relieved the inhibition of electron flow from H_2O to DCIP in Co^{2+}-pretreated chloroplasts. Addition of $MnCl_2$ to washed chloroplasts resulted in no alleviation of Co^{2+} inhibition of the PS II reaction.

The above results demonstrate that like Zn^{2+} [8], Co^{2+} also inhibits electron flow catalyzed via both photosystems (Figs. 1 and 3), although a higher concentration of $CoSO_4$ (8 mM) than $ZnSO_4$ (2 mM) was required to bring about similar extent of inhibition [8]. Even a low concentration of $CoSO_4$ (2 mM) exerted about 40% inhibition on the whole-chain electron flow (Figs. 1 and 3). Co^{2+}-induced inhibition of PS II-catalyzed electron flow from H_2O to DCIP was affected approximately to the same extent as that of whole-chain electron flow (Figs. 1 and 2). Co^{2+} has no effect on the PS I-mediated electron flow from $DCIPH_2$ to methyl viologen (Fig. 3) while incubation of chloroplasts with $ZnCl_2$ affects PS I activity [8]. Thus, it is clear that like Zn^{2+} [8], Co^{2+} mostly affects PS II activity of isolated chloroplasts. However, unlike Zn^{2+}-inhibition [8], Co^{2+}-induced inhibition of PS II activity (Hill activity or lowering of Chl *a* fluorescence yield) could

neither be relieved by the supply of an exogenous donor such as NH_2OH nor could the inhibitory effect be removed by washing chloroplasts with buffer (Fig. 6). Thus, Co^{2+} treatment seems to affect irreversibly the PS II pigment system of chloroplasts. The lowering of Chl a fluorescence intensity by Co^{2+} treatment of chloroplasts (Figs. 4 and 6), at first glance, suggests that Co^{2+} inhibits electron flow in a manner similar to Tris washing of chloroplasts [24]. The latter treatment inactivates O_2 evolution and thus acts as a block on the electron donor side of the PS II electron-transport chain. However, in Co^{2+}-treated chloroplasts, the loss of Chl a fluorescence intensity could not be relieved by the addition of NH_2OH, which suggests that Co^{2+} probably directly affects the PS II reaction centre and thus that Co^{2+} inhibition of photoelectron transport is mostly irreversible.

Thus, it appears that Co^{2+} acts very close to the PS II reaction centre complex so that even NH_2OH fails to donate electrons to PS II. This is in contrast to the action of Zn^{2+} which only blocks electron flow from H_2O to PS II [8]. The exact mode of inactivation of PS II function by Co^{2+} remains to be investigated.

Acknowledgements

References

1 Bazzaz, M.B. and Govindjee (1971) Environ. Lett. 6, 171–191
2 Miles, C.D., Brandle, J.R., Daniel, D.J., Chu-Dor, O., Schnare, P.D. and Uhlik, D.J. (1972) Plant Physiol. 49, 820–825
3 Cedeno-Maldonado, A., Swader, J.A. and Heath, R.L. (1972) Plant Physiol. 50, 698–701
4 Kimimura, M. and Katoh, S. (1972) Biochim. Biophys. Acta 283, 279–292
5 Bazzaz, M.B. and Govindjee (1974) Environ. Lett. 6, 1–12
6 Li, E.H. and Miles, C.D. (1975) Plant Sci. Lett. 5, 33–40
7 Wong, D. and Govindjee (1976) Photosynthetica 10, 24–25
8 Tripathy, B.C. and Mohanty, P. (1980) Plant Physiol. 66, 1174–1178
9 Shrotri, C.K., Rathore, V.S. and Mohanty, P. (1981) J. Plant Nutr. 3, 945–955
10 Manes, M.O. and Keppas, A. (1975) J. Biol. Chem. 250, 4171–4178
11 Demattes, F. and Gibbs, A.H. (1977) Biochem. J. 162, 213–216
12 Agarwala, S.C., Bishit, S.S. and Sharma, C.P. (1977) Can. J. Bot. 55, 1299–1307
13 Wilder, G.F. and Henkel, J. (1979) Planta 146, 223–228
14 Biswal, U.C., Singhal, G.S. and Hohanty, P. (1979) Ind. J. Exp. Biol. 17, 262–264
15 Tripathy, B.C. (1981) Ph.D. Thesis, Jawaharlal Nehru University, New Delhi, India
16 Sun, A.S.K. and Sauer, K. (1973) Biochim. Biophys. Acta 234, 399–414
17 Hauska, G. (1977) in Encyclopedia of Plant Physiology (Trebst, A. and Avron, M., eds.), Vol. 5, pp. 253–265, Springer-Verlag, Berlin
18 Izawa, S. (1977) in Encyclopedia of Plant Physiology (Trebst, A. and Avron, M., eds.), Vol. 5, pp. 266–282, Springer-Verlag, Berlin
19 Govindjee and Govindjee, R. (1975) in Bioenergetics of Photosynthesis (Govindjee, ed.), pp. 1–50, Academic Press, New York
20 Goedheer, J.C. (1972) Annu. Rev. Plant Physiol. 23, 87–112
21 Butler, W.L. and Katajima, M. (1975) Biochim. Biophys. Acta 396, 72–85
22 Kouchbe, S.M. (1980) Photosynthetica 14, 8–11
23 Govindjee and Jursinic, P. (1979) in Photochmistry and Photobiology Review (Smith, K., ed.), Vol. 4, pp. 3–205, Plenum Press, New York
24 Yamashita, T. and Butler, W.L., (1968) Plant Physiol. 43, 1978–1985
25 Mohanty, P., Braun, B.Z. and Govindjee (1972) FEBS Lett. 20, 273–276
26 Mohanty, P., Mayne, B. and Ke, B. (1979) Biochim. Biophys. Acta 545, 285–295