Volume : 2 | Issue : 3 | March 2013 • ISSN No 2277 - 8160

Research Paper | Agriculture

nthetic Electron Transport in Chloroplast of Higher Cells: Evidence with Electron Spin Resonance (Esr) Spectroscopy

Santosh Kumari | Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi 110012. India

ABSTRACT

Flash induced primary charge separation was detected as photosystem1 (PS1) signal by ESR in intact leaf discs and isolated chloroplasts of spinach (Spinacia oleracea L.) and 'Morden' cultivar of sunflower (Helianthus annuus L.). PS1 signal was enhanced after incubation of samples in increasing concentrations i.e. 0.5M and 1M concentrations of potassium cyanide (KCN) and sodium cyanide (NaCN). Our data indicate that a respiratory electron transport pathway involving a cyanide sensitive component is located in chloroplasts and competes with photosynthetic electron transport for reducing equivalents from the plastoquinone pool.

KEYWORDS: chloroplast, leaf, Spinacia, Helianthus, Electron spin resonance, Chlororespiration

Introduction

The interaction between photosynthesis and respiration is an enigma as referred by Gyozo et al.and stated by Graham and Chapman, particularly in higher plants. However, the interaction between the two processes has received little attention. Godde and Trebst (19800 found evidence of the existence of NADPH dehydrogenase in Chlamydomonas reinhardii. A respiratory pathway for the oxidative degradation of starch had been reported (Glfeller and Gibbs, 1985) in chloroplast. Reducing equivalents generated during the degradation of starch were shown to enter the plastoquinone pool in Chlamydomonas. Most photosynthetic bacteria develop both respiratory and photosynthetic electron transport chains on the same membrane. Respiratory and photosynthetic electron transport chains share components, such as the membrane bound cytochrome b/f complex (Peschek, 1983). Plastoquinol-cytochrome b/f reductase has been shown to be a common electron donor to P700 and the respiratory oxidase, with oxygen regulating the diversion of reducing equivalents from the photosynthetic electron transport chain (Matthijs et al., 1984). Flash mass spectrometry in green algal cells indicated that PS1 activity can divert electrons from the chlororespiratory chain in a manner similar to that found in photosynthetic bacteria (Peltier et al. 1987).

In this paper, experimental data are presented which strongly indicate the existence of chlororespiration in higher plant cells and isolated intact chloroplasts that is very similar to the respiratory electron-transport section operating in photosynthetic bacteria, cyanobacteria and green algae.

Materials and methods

Chloroplast Isolation

Chloroplasts were prepared from local market spinach (Spinacia oleracea L.) and third leaf of sunflower (Helianthus annuus L.). Deveined leaves were ground for 20s with a blender in a medium containing sorbitol (350 mM), MgCl2 (5 mM), NaCl (10 mM), 2-Mercaptoethanol (5 mM) and 30 mM Tris-HCl (pH 7.5).

The slurry was filtrated through two layers of cheese cloth and filterate was centrifuged at 200g for 1 min, pallet was discarded. Supernatant was centrifuged at 3000g for 2 min and the resultant chloroplast pellet was dispersed in the same buffer at the concentration of 15Chl/ml. All steps were performed at 4°C. The concentration of chlorophyll a was determined according to Arnon (1949). The samples having a concentration of approx. 5 mg Chla/ml were placed into flat cuvettes of ER 200E/D spectrometer (Bruker, Germany) using X-band. The samples were illuminated directly in the resonator using an ultraviolet radiation lamp. Console configuration-ER 211, and ER 073 10" Magnet.

Results

In the dark adapted leaf or chloroplast, we obtained an ESR signal of PS11 and upon illumination with ultraviolet (UV-entire range) lamp; a signal of PS1 was recorded. Figure (1B, C, D, E) shows that KCN activated the PS1 reaction centres. The enhancement was clearly detected with the first flash following the addition of KCN. The fact, however, that KCN did induce an enhancement even with a flash of light for < 2 seconds, indicates that processes other than competition between the two redox chains may have to be taken into account. Reactivation of the 'Silent' reaction centres by cyanide can be complex and cannot be simply explained by alterations in the redox state of the plastoquinone pool. Amplitude in isolated chloroplast membranes was unaffected by 1 mM KCN. PS1 signal was enhanced unto 500 mM KCN in our experiments. PS1 signal indicated that a large number of reaction centres were inactive upon flash excitation, and KCN reactivated the silent reaction centres. Such results were obtained in leaf discs of sunflower and spinach, demonstrating that enhancement in isolated chloroplasts is not the consequence of some artifact introduced by isolation procedures. Furthermore, a similar enhancement in PS1signal occurred in the presence of NaCN in these leaf discs. These results substantiate the conclusion on the presence of respiratory control of chloroplasts in higher plant cells. In the presence of ascorbate and light there was no enhancement in the PS1 signal compared to control (Fig 1G, H). This indicates that ascorbate, despite the fact that it provides sufficient redox power for PS1, could not fully restore the activity of this photosystem. This experimental observations calls attention to the fact that reactivation of the 'Silent' PS1 centres by cyanide can be complex and cannot be simply explained by alteration in the redox state of plastoquinone pool. The available high number of reducing equivalents for the plastoquinone pool and the rapid PS1 donors may render the respiratory pathway unnoticeable.

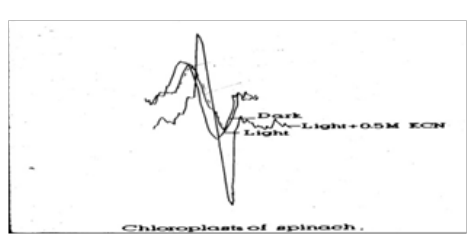
Addition of KCN to chloroplast preparations obtained from sunflower and spinach showed a quantitatively similar and substantial enhancement of PS1 signal.

A

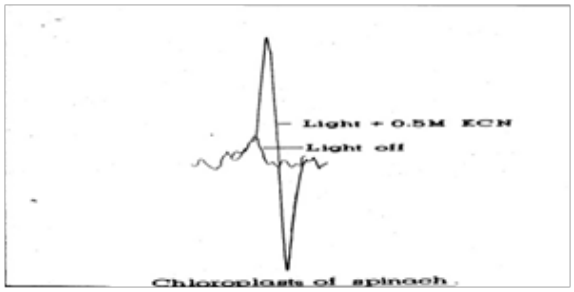
C



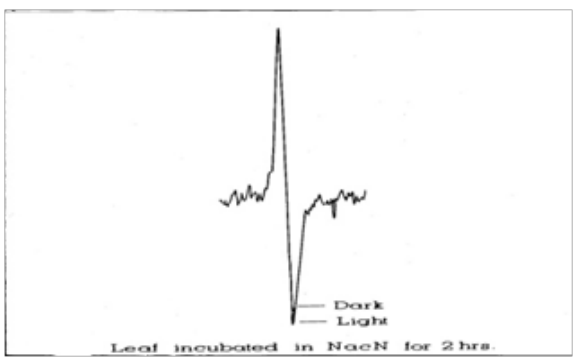
B



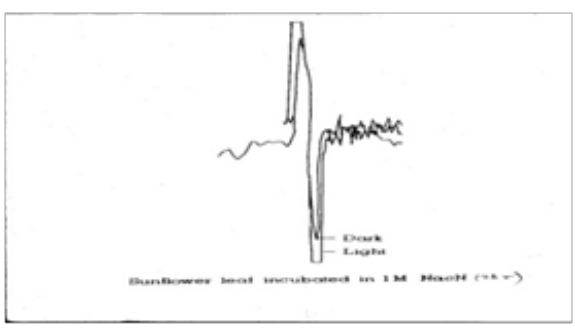
C



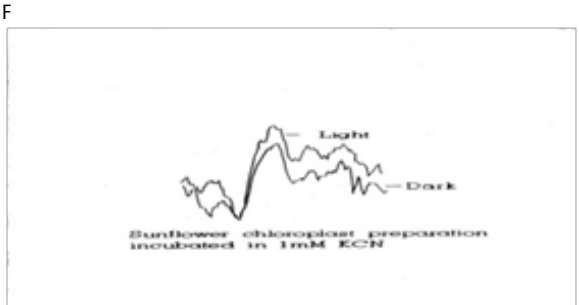
D



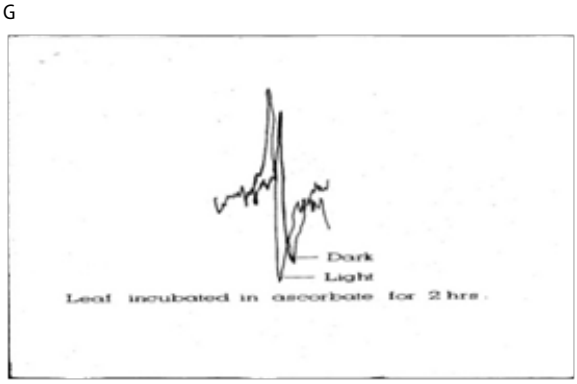
E



F



G



H

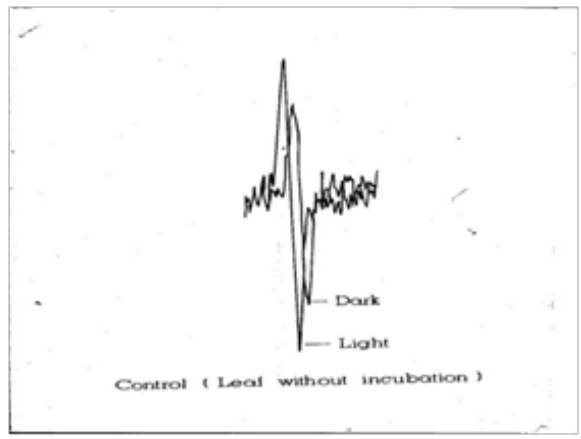


Fig 1. Characteristic Spinach chloroplast signal recorded at 30 mW, 1.26 mW and 12.6 mW of microwave power (A) for PSI (A) g= 1.99 ΔHpp= 19 Gauss; Field modulation intensity= 5 x 1 Gpp; Gain 6.3x105; Microwave frequency= 9.68 , PSII signal in dark; in light and On illumination of chloroplasts after incubated in 500 mM potassium cyanide for 2 hours (B; C), Incubated in 500 mM Sodium cyanide for 2 hours, g= 2.027, ΔHpp =3.75 Gauss; Field modulation intensity= 5 x 1 Gpp; Microwave frequency= 9.79 ; Cavity TE 102; Microwave power 20 dB; Mid range 3480 G; scane range 500 G; Scan time 100 seconds at Room temperature; Phase 90°.

Sunflower leaf pieces incubated in 1M Sodium cyanide for 2 hours (E) g= 2.027, ΔHpp =3.75 Gauss; Field modulation intensity= 1.25 x 1 Gpp; Microwave frequency= 9.80 ; Cavity TE 102; Microwave power 20dB; Mid range 3480 G; scane range 500 G; at Room temperature; Phase 90°. Sunflower chloroplast preparation incubated in 1mM potassium cyanide (F) ; g= 2.31; ΔHpp 13.2 Gauss; modulation intensity 4x1; Gain 2x106 . Sunflower leaf pieces incubated with 50 mM Ascorbate (G) at modulation intensity 4x1 Gpp and leaf pieces without any treatment/ control (H)

Discussion

The experimental results demonstrate that photosynthetic electron transport in higher chloroplast interact with a respiratory pathway. Presence of PS11 in dark showed the continuous supply of electons to PS1 and absence of PS1 signal indicated the presence of chlororespiration. The fraction of PS1 reaction centres turning over can be estimated from the value of light-dark signal in the absence of 3-(3', 4' dichlorophenyl)-1, 1 dimethylurea (DCMU) and directly with illumination in the presence of DCMU (Chetverikov 1983). The activity of PS1 in the leaf discs (sunflower) is retained to a certain extent after the addition of DCMU and continuous illumination of samples with UV light. This indicated that there is a continuous supply of reducing equivalents for PS1. If respiration competes for these electrons they would not be available for PS1. Upon blocking respiration by KCN, these electrons should be directed from respiratory to photosynthetic electron transport and thus enhance the photochemical process. Respiratory inhibitor KCN increased the PS1 signal and reactivation of reaction centres. The amplitude of the signal was considerably larger than that recorded in control. KCN improved the turnover of cytochrome b/f complex (Garab et al.1989) and flash induced absorbance transients of cytochrome b/f complex. In the absence of cyanide electrons from the cytochrome b/f complex may be diverted from photosynthetic electron transport, thus rendering a fraction of reaction centres 'Silent'. A similar regulatory mechanism, competition for electrons between photosynthesis and respiration has been shown to function in photosynthetic bacteria (Vermeglio and Joliot, 1984) and in green algal cells (Peltier et al., 1987).

With increasing concentrations of KCN, the partial inhibition of the electron transfer between plastocyanin and P700+ was progressively decreased in our data are in confirmation with earlier reports (Izawa, 1977; Garab et al. 1989). The distinction between chlororespiration and the Mehler reaction was achieved by using PS1 deficient algal cells (Bennoun, 1982). Reduction of oxygen via the PS1 acceptor side is strictly a light dependent reaction. Enhancement of signal by KCN,

however, was observed by a flash of light within seconds. The respiratory interaction occurs on the donor side of PS1, at the cytochrome b/f complex and at the plastoquinone pool rather than at the acceptor side of PS1 (Garab et al. 1989). Saturation of the reactivation of 'Silent' reaction centres were reported at 5 mM KCN (Garab et al. 1989). Cyanide is a respiratory poison; its toxicity is based upon a high affinity for the ferric heme form of cytochrome a3 (cytochrome oxidase). In addition, cyanide also affects photosynthesis, through formation of a stable complex with ribulose-1, 5-bisphosphate carboxylase/oxygenase (Wisnik and Lane, 1969) or inhibition of plastoquinone-oxidoreductase (Buchel and Garab, 1995). Chalker & Taylor (1975) and Barnes (1985) reported that photosynthetic oxygen evolution in Acropora cervicornis and A. formosa is reduced following exposure to 10-5 M NaCN.

The cyanide sensitive terminal oxidase is most likely found in the stroma or the envelope membrane of chloroplasts, as indicated by its loss upon isolation of thylakoid membranes from intact chloroplasts. Cytochrome b560 had been suggested to participate in chlororespiration (Rolfe et al. 1987). A functional interaction of the two organelles may explain their linkages. Five sequences coding for proteins homologous to components of the mitochondrial respiratory chain NADH dehydrogenase have been found in tobacco chloroplast (Shinozaki et al. 1986). Under stress conditions, other roles of these components might superimpose to the energy balance role. In particular, NDH complex and chlororespiration activities would help avoiding over-reduction of PSI electron acceptors, scavenging reactive oxygen species (ROS), therefore, protecting PSI from photoinhibition. However, these chloroplast functions do not operate independently from the cellular context, and in particular, the occurrence of several redox exchange shuttles between chloroplasts and mitochondria (Hoefnagel et al. 1998) allows the mitochondria to participate also in redox and energetic balance of the chloroplast. Respiratory and photosynthetic chains may be under thermodynamic control by transmembrane electrochemical gradient (Vermeglio and Joliot, 1984). Regulation of photosynthesis by chlororespiratory functions which all have their specificities and time constants (Cournac et al. 2002), eventually cooperate towards acclimation of photosynthesis in natural environment.

Our data indicate that there is a cyanide sensitive component in the chloroplast that diverts the electrons to photosystem1 when chlororespiration is inhibited with KCN. Unravelling the roles of chlororespiratory components will help in assessing how and in which environmental conditions they contribute to plant fitness.

REFERENCES

Barnes, D. J. (1985). The effects of photosynthetic and respiratory inhibitors upon calcification in the staghorn coral Acropora formosa. Proc 5th Int Coral Reef Congr 6:161-166. Bennoun, P. (1982). Evidence for a respiratory chain in the chloroplast. Proceedings of the National Academy of Sciences of the USA 79, 4352–4356. Buchel, C., & Garab, G. (1995). Evidence for the operation of a cyanide-sensitive oxidase in chlororespiration in the thylakoid of the chlorophyll c containing alga Pleurichloris meiringensis (Xanthophyceae). Planta 197.69-75 Chalker, B. E., & Taylor, D. L. (1975). Light-enhanced calcification and the role of oxidative phosphorylation in calcification of the coral Acropora cervicornls. Proc R Soc Lond B Biol Sci 190:323-331 Chetverikov, A. G. (1983). Use of EPR spectroscopy to study the physiology of photosynthesis. Fiziologiya Rastenii 30: 682-692. Cournac, L., Latouche, G., Cerovic, Z., Redding, K., Ravenel, J. & Peltier, G. (2002). In vivo interactions between photosynthesis, mitorespiration, and chlororespiration in Chlamydomonas reinhardtii. Plant Physiol. 129, 1921–1928. Garab, G., Lajkó, F., Mustárdy, L. & Márton, L. (1989). Respiratory control over photosynthetic electron transport in chloroplasts of higher-plant cells: evidence for chlororespiration. Planta 179: 349-358. Godde, D., & Trebst, A. (1980). NADH as electron donor for the photosynthetic membrane of Chlamydomonas reinhardii .Arch. Microbiol. 127:245-252. Gfeller, R. P., & Gibbs, M. (1985). Fermentative metabolism of Chlamydomonas reinhardii III. Role of plastoquinone. Plant Physiol. 77: 509-511. Hoefnagel, M. H. N., Atkin, O. K. & Wiskich, J. T. (1998). Interdependence between chloroplasts and mitochondria in the light and the dark. Biochem. Biophys. Acta 1366: 235–255. Izawa, S. (1977). Inhibitors of electron transport. In: Encyclopedia of plant physiology, N.S., vol5: Photosynthesis I, pp. 266-282. Trebst A., Avron M. eds. Springer, Berlin Heidelberg, New York Malthijs, H. C. P., Luderus, E. M. E., Scholts, M. J. C. & Kraayenhof, R. (1984). Energy metabolism in the cyanobacterium Plectonema boryanum. Oxidative phosphorylation and respiratory pathway. Biochim. Biophys. Acta 766: 38-44. Peltier, G., Ravenel, J., & Vermeglio, A. (1987). Inhibition of respiratory activity by short saturating flashes in Chlamydomoas: evidence for chlororespiration. Biochem. Biophys. Acta 893: 83-90. Peschek, G. A. (1983). The cytochrome f-b electron transport complex. A common link between photosynthesis and respiration in the cyanobacterium Anacystis nidulans. Biochem. J. 210: 269-272. Rolfe, S. A., Sanguansermsri, M., Bendall, D. S. (1987). Cytochrome b 560, a new component of thylakoid membranes. Biochem. Biophys. Acta 894: 434-442. Rumeau, D., Peltier, G. & Cournac, L. (2007). Chlororespiration and cyclic electron flow around PSI during photosynthesis and plant stress response Plant, Cell and Environment 30, 1041–1051. Shinozaki, K., Ohme, M., Tanaka, M., Wakasugi, T., Hayashida, N., Matsubayashi, T., Zaita, N., Chunwongse J., Obokata, J., Yamaguchi-Shinoyaki, K., Ohto, C., Torazawa, K., Meng, B.Y., Sugita, M., Deno, H., Kamogashira T., Yamada K., Kusuda J., Takaiwa F., Kato A., Tohdon N., Shimada H., & Sugiura, M. (1986). The complete nucleotide sequence of the tobacco chloroplast genome: its gene organization and expression. EMBO J. 6: 2043-2049. Vermeglio, A., & Joliot, P. (1984). Light induced absorption changes in intact cells of Rhodopseudomonas sphaeroides. Evidence for interaction between photosynthetic and respiratory electron transport chains. Biochem. Biophys. Acta 764: 226-232. Wishnik, M, & Lane, M. D. (1969). Inhibition of ribulose bisphosphate carboxylase by cyanide inactive ternary complex of enzyme ribulose diphosphate carboxylase by cyanide. J Biol Chem. 244: 55-59.