PERGAMON

Phytochemistry 50 (1999) 203–208

PHYTOCHEMISTRY

NADPH and ferredoxin:NADP$^+$ oxidoreductase-dependent reduction of quinones and their reoxidation☆

Monika Bojko, Stanisław Wieckowski *

Department of Physiology and Biochemistry of Plants, The J. Zurzycki Institute of Molecular Biology, Jagiellonian University, al. Mickiewicza 3, 31-120 Krakow, Poland

Revised 22 July 1998

Abstract

Molecular oxygen uptake was initiated by adding NADPH (1 mM) to the buffered medium containing 0.6 μM spinach ferredoxin:NADP$^+$ oxidoreductase and 20 μM quinone (plastoquinone-2, decyl-plastoquinone, decyl-ubiquinone, or duroquinone). At pH 7.7 the rate of oxygen uptake was 2- to 12-fold higher during an initial phase (V_1) than in a subsequent phase (V_2). Except for duroquinone, the initial rate of oxygen consumption was ca. 2.7-fold higher in alkaline than in acidic medium. Ferredoxin was not essential, although it stimulated the reaction investigated. Oxygen uptake was not detectable with plastoquinone-9 or α-tocoquinone. The possible mechanisms of the NADPH and ferredoxin:NADP oxidoreductase dependent reduction of some quinones and their reoxidation are discussed.

Keywords: Decyl-plastoquinone; Decyl-ubiquinone; Diaphorase activity; Dibromothymoquinone; Duroquinone; Ferredoxin:NADP$^+$ oxidoreductase; Oxygen uptake; Plastoquinone-2; Plastoquinone-9; α-Tocoquinone

1. Introduction

Chloroplastic ferredoxin:NADP$^+$ oxidoreductase (EC 1.18.1.2, FNR) is involved in the last step of the electron transport chain catalysing NADP$^+$ reduction by electrons derived from reduced ferredoxin (for recent review see Arakaki, Ceccarelli, & Carrillo, 1997). It has also been well established that in vitro this enzyme exhibits diaphorase activity that catalyses NADPH-dependent reduction of ferricyanide, dichlorophenol indophenol, tetrazolium salts (Zanetti & Forti, 1966; Forti & Sturani, 1968; Zanetti, 1981), cytochrome c (Forti & Sturani, 1968), methyl viologen (Bowyer, O'Neill, Camilleri, & Todd, 1988), non-physiological quinones (Anusevicius, Martinez-Julvez, Genzor, Nivinskas, Gomez-Moreno & Cenas, 1997) and some other artificial electron acceptors (for review see Carrillo & Vallejos, 1987). It can also be involved in the NADPH-dependent reduction of NAD$^+$ or its analogues (Böger, 1971). It is still an open question whether diaphorase activity of FNR plays any role in vivo in the oxidation of NADPH. Some evidence has also been published for participation of this enzyme in cyclic electron transport around photosystem 1 (Moss & Bendall, 1984; Hosler & Yocum, 1985). However, the mechanism of FNR-catalysed step(s) of cyclic electron transport in chloroplasts of higher plants is still under debate (Bendall & Monasse, 1995; Arakaki et al., 1997). One could assume that FNR mediates electron flow from reduced Fd to other electron acceptors, like cytochrome b_6/f complex and/or plastoquinone pool (Bendall & Monasse, 1995). We have previously found (Bojko & Wieckowski, 1995) that dibromothymoquinone (2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone, DBMIB) is a good electron acceptor in the diaphorase activity of FNR. This suggests that reduction of plastoquinone may be mediated by FNR. In this study we attempted to answer the question whether FNR can catalyse the NADPH-dependent reduction of plastoquinone and some other quinones which differ from one another in the side chains and to a lesser extent in the reducible heads. We have also demonstrated (Bojko & Wieckowski, 1995) that reduced DBMIB is reoxidized by molecular oxygen

☆ Preliminary results were presented at the 10th FESPP Congress, Florence, Italy (Plant Physiol. Biochem., Special issue, 1996, p. 99).

* Corresponding author. E-mail: wieckowski@mol.uj.edu.pl.

0031-9422/98/$ - see front matter
PII: S0031-9422(98)00540-8

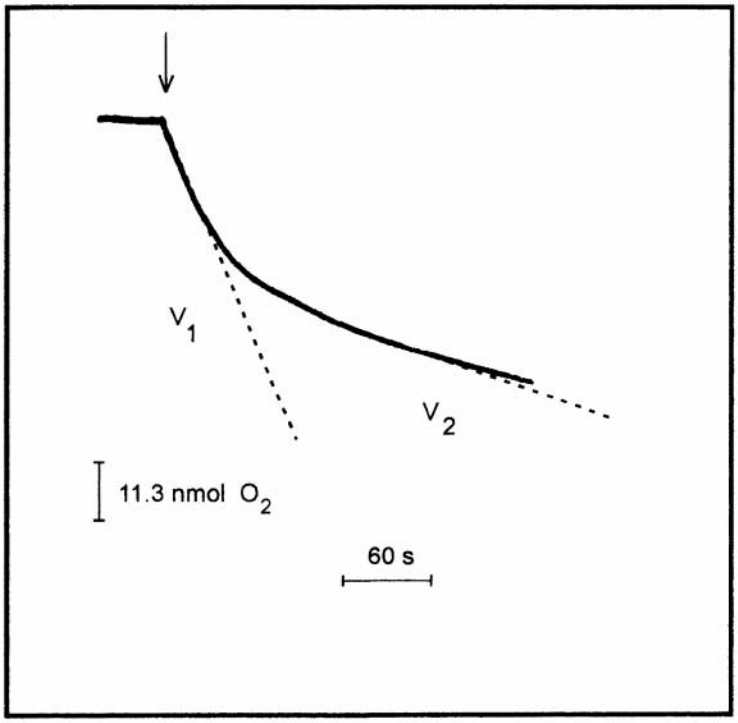

Fig. 1. Typical record of NADPH (1 mM)-initiated oxygen uptake by the buffered (pH 7.7, 40 mM Tris–HCl) reaction mixture (1 cm^3) containing 1 mM Na_2EDTA, 1 mM $MgCl_2$, 0.6 μM FNR and 20 μM duroquinone (or other quinones). V_1, rate of oxygen uptake during the 1st minute of measurement; V_2, rate of oxygen uptake between 2nd and 3rd minutes of measurement.

and this process can be conveniently followed by monitoring oxygen uptake.

2. Results and discussion

Adding NADPH to the buffered reaction mixture of FNR and DBMIB caused oxygen uptake which proceeded in one or two phase kinetics (Bojko & Wieckowski, 1995). Oxygen consumption was associated with the reoxidation of reduced quinone. Under anaerobic conditions the pseudo-first-order rate constant (k) and the Michaelis constant (K_m) for NADPH- and FNR-dependent DBMIB reduction were 0.34 s^{-1} and 16 μM, respectively. The data presented in this paper clearly show that the uptake of molecular oxygen was also induced by adding NADPH to the reaction medium containing FNR and PQ-2, decyl-PQ, decyl-UQ or duroquinone, but not to that with PQ-9 or α-TQ. In most experiments these rates were higher in an initial (V_1) than in a subsequent step (V_2) (Fig. 1). Fig. 2 shows that under conditions applied the highest V_1 was supported by duroquinone (0.058 μmol O_2 per min) with decreasing rates in the order PQ-2 (0.031 μmol O_2 per min), DBMIB (0.026 μmol O_2 per min), decyl-UQ (0.013 μmol O_2 per min) and decyl-PQ (0.007 μmol O_2 per min). V_1 was 2- to 12-fold higher than V_2 values and the highest V_2 value was with PQ-2 (0.013 μmol O_2 per min). No relationship between V_1 and V_2 could be established. The two phases of oxygen uptake may be interpreted in the terms of sequences of quinone reductions, e.g. ubiquinone-10 in protic medium may occur in six states of oxidation and protonation (Morrison, Schelhorn, Cotton, Bering, & Loach, 1982). Quinols interact rather slowly with molecular oxygen (Kruk, Jemioła-Rzeminska, & Strzałka, 1997) whereas mono- and di-valent anions or semiquinones are very strong reductants (Sugioka et al., 1988). This suggests that V_1 is associated with the oxygen reduction by quinone anions and/or semiquinones, whereas V_2 may be connected with the oxygen reduction by quinols.

The effects of pH on the NADPH dependent reduction of PQ-2 (Fig. 3) and decyl-PQ (Fig. 4) and their reoxidation by molecular oxygen are comparable with those observed for DBMIB (see also (Bojko & Wieckowski, 1995)). In both cases the V_1 values were ca. 2.7-fold higher in pH 8.7 than in pH 6.7. Ferredoxin (Fd) was not an essential component for the reaction investigated, however, at pH 7.7 it stimulated oxygen uptake about 1.6-fold with PQ-2 and 2.2-fold with decyl-PQ. No clear dependence of V_2 values on the pH was found. On the other hand the pH's 6.7–8.7 had no effect on the rate of NADPH stimulated oxygen uptake in the presence of duroquinone

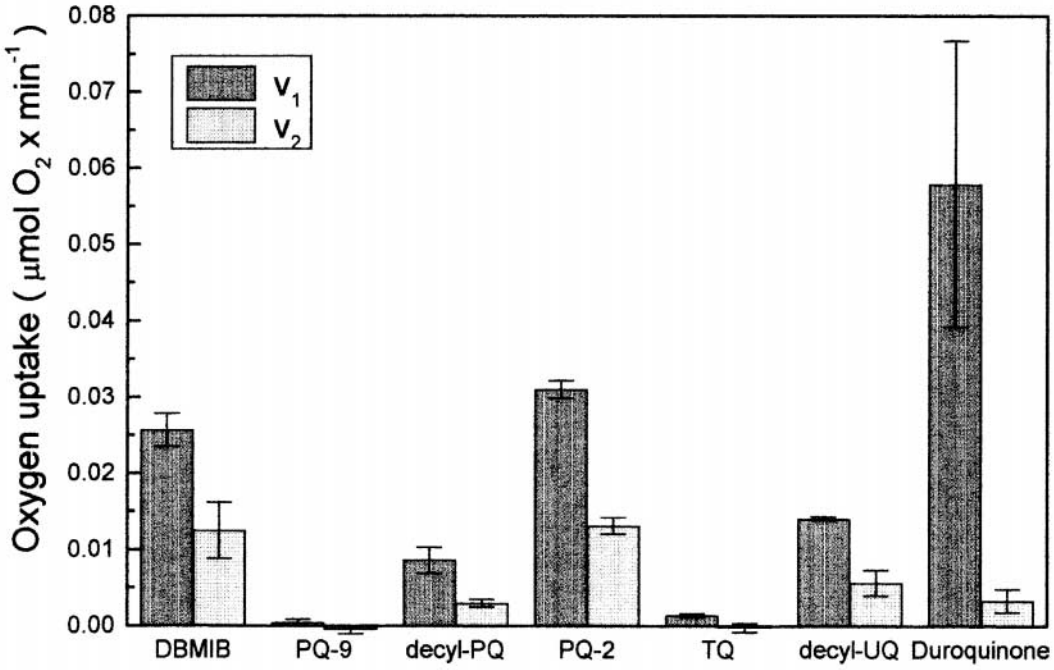

Fig. 2. NADPH (1 mM) initiated oxygen uptake (V_1, V_2 values) by the reaction mixture containing also 0.6 μM FNR and 20 μM quinone as indicated. Other details as in Fig. 1. DBMIB, dibromothymoqinone; PQ-9, plastoquinone-9; decyl-PQ, decyl-plastoquinone; PQ-2, plastoquinone-2; α-TQ, α-tocoquinone; decyl-UQ, decyl-ubiquinone.

(Fig. 5). In this case the V_1 values were high (about 0.055 μmol O_2 per min) at all three pHs investigated and V_2 values were highest in alkaline pH. Ferredoxin had no stimulatory effect on this process. The dependence of oxygen uptake upon pH confirms the assumption that semiquinones or semiquinone anions are involved efficiently in O_2 reduction because higher pH (with a domination of unprotonated phenolic groups) favored a greater speed of oxygen uptake as compared with that in lower pHs (see also Sugioka et al., 1988). Semiquinones should be rather stable in pH 7.7 and 8.7 because their pK_a values are in the range 3.5–5.0

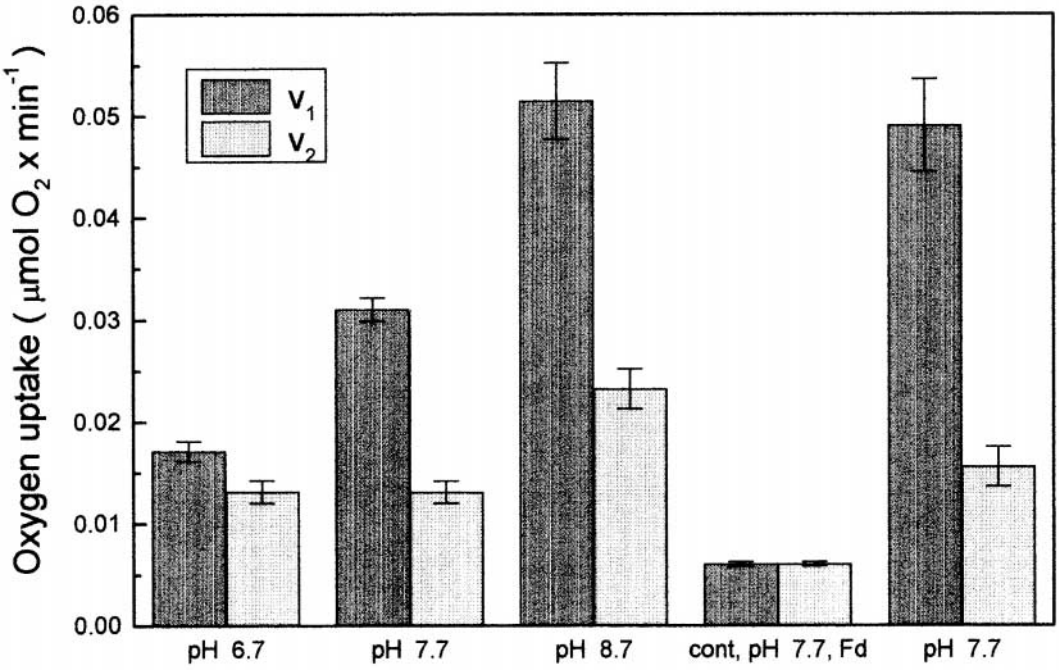

Fig. 3. Effects of pH (6.7, 7.7, 8.7) or ferredoxin, Fd (0.6 μM, as indicated) on the NADPH (1 mM) initiated oxygen uptake in the reaction mixture containing 0.6 μM FNR and 20 μM PQ-2. For the composition of the basic reaction mixture and other details see Fig. 1.

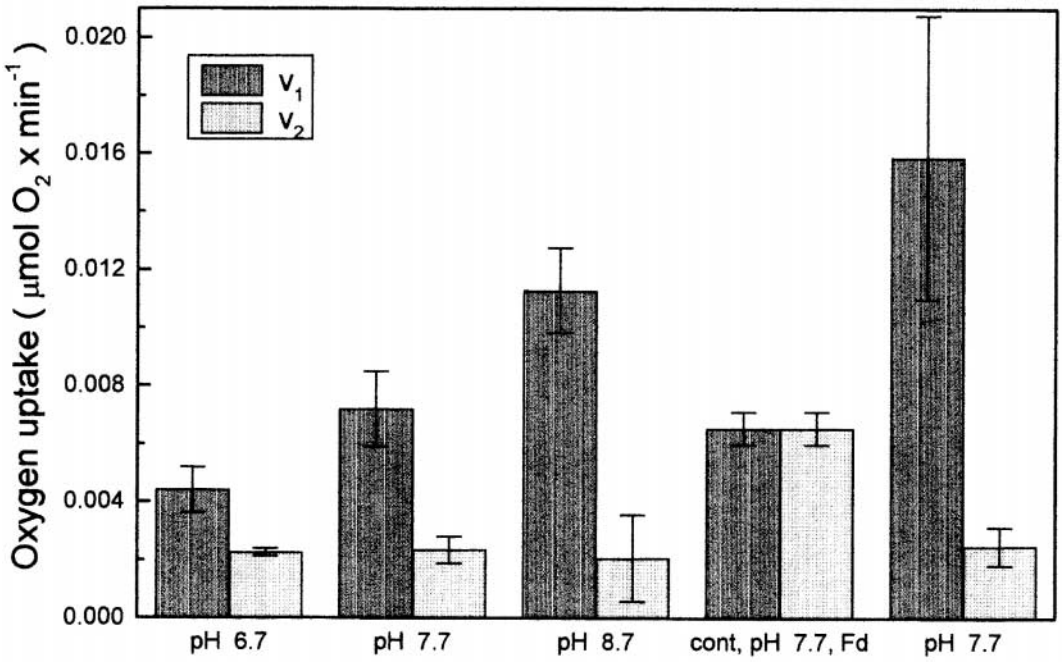

Fig. 4. The effects of pH (6.7, 7.7, 8.7) or Fd (0.6 μM, as indicated) on the NADPH (1 mM) initiated oxygen uptake by the reaction mixture containing 0.6 μM FNR and 20 μM decyl-PQ. For composition of the basic reaction mixture and other details see Figs. 1 and 2.

(Swallow, 1982) whereas those for many hydroquinones range from 8.0 to 13.3 (Morrison et al., 1982). Higher activity of FNR in alkaline medium (Batie & Kamin, 1981) should be also taken into account. Independence of oxygen uptake in the presence of duroquinone on the pH suggests that this quinone is unprotonated in the pHs 6.7 and 8.7.

The FNR- and NADPH-dependent reduction of PQ-9 and α-TQ were also not detectable under anaerobic conditions (data not shown). Since PQ-9 and α-TQ differ mainly from the other investigated quinones in the structure of their non-reducible side chains, the results obtained could be explained by assuming that any binding site(s) on FNR molecules is not accessible

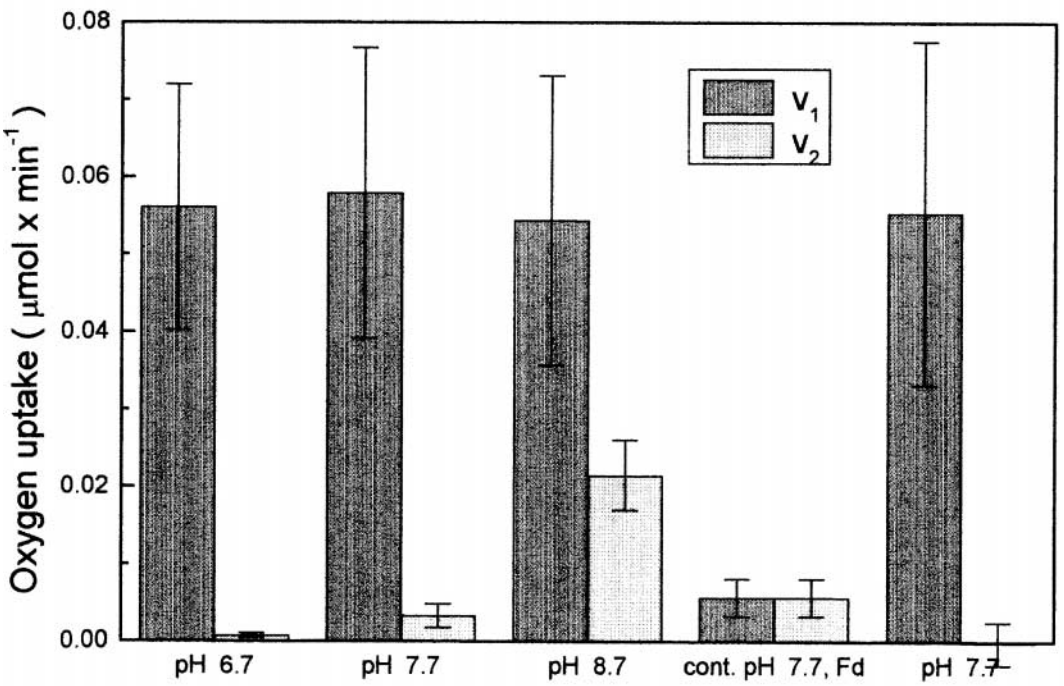

Fig. 5. Effect of pH (6.7, 7.7, 8.7) or Fd (0.6 μM, as indicated) on the NADPH (1 mM) initiated oxygen uptake by the reaction mixture containing 0.6 μM FNR and 20 μM duroquinone. For composition of the basic reaction mixture and other details see Figs. 1 and 2.

to the reducible groups of these two molecules. It is likely that the long side chains and high hydrophobicity prevent the interaction of PQ-9 and α-TQ molecules with FNR. It is known that PQ-9 exhibits extremely low solubility in polar solvents compares with the other quinones. In confirmation of this we did not observe for PQ-9 suspension a 2-nm shift of the absorption peak towards the shorter wavelength, but this shift was observed for the other quinones investigated (data not shown). Probably PQ-9, like ubiquinone-10 (Lenaz, Espost, & Castelli, 1982), remained in our reaction medium in the form of micelles resemble those of Triton x-100. The quinones, other than PQ-9 and α-TQ, are partially soluble in polar medium and it is likely that this enables them to interact with active site(s) on FNR. The results obtained confirmed the suggestion of some authors (Shahak, Crowther, & Hind, 1981; Ravenel, Peltier, & Havaux, 1995) that under in vivo conditions FNR may be involved directly in the plastoquinone pool reduction. It is likely that localization of PQ-9 molecules in thylakoid membranes prevents its micellization and favors its head interaction with FNR. The accessibility of some quinones, including PQ-9, in unbroken thylakoid membranes to specific antibodies (Radunz & Schmid, 1973; Schmid et al., 1996) confirms this hypothesis. Additional proteins, e.g. PSI-E subunit (Nielsen, Andersen, & Scheller, 1995) and/or *ndh*B and *ndh*J gene products (Guedeney, Corneille, Cuine, & Peltier, 1995) are thought to be associated with FNR molecules and may also favor PQ-9 reduction in thylakoid membranes. The life times for mono- and/or divalent PQ-9 anions, if they occur in vivo, should be very short because efficient oxygen uptake associated with PQ-9 autooxidation has not been revealed. One can consider that the quinone binding site on FNR corresponds to that for 2,6-dichlorophenol indophenol (DCPIP) (Lenaz et al., 1982) and it differs from high affinities Fd or $NADP^+$ (NADPH) binding sites since eleven monoclonal antibodies raised against spinach FNR blocked specifically only the DCPIP reduction (Chang, Morrow, Hirasawa, & Knaff, 1991). The $NADP^+$ and Fd binding sites have been well defined in the 1.7 Å resolution structure (Bruns & Karplus, 1995) whereas that for DCPIP has not yet been recognized in crystallographic studies. If we assume that the DCPIP (plastoquinone?) binding site is also accessible to electrons from Fd_{red} no hypothetical ferredoxin:-plastoquinone reductase (Bendall & Monasse, 1995) is needed for explanation of the mechanism of plastoquinone pool reduction in cyclic electron flow around photosystem 1 (for review see also Wieckowski & Bojko, 1997).

3. Experimental

Reagents: plastoquinone-2 and plastoquinone-9, were gifts from Hoffman-La Roche, decyl-plastoquinone and decyl-ubiquinone were purchased from Sigma and α-tocoquinone was from Merck. Dibromothymoquinone was obtained from Aldrich. The reagents for chromatography were purchased as indicated previously (Bojko & Wieckowski, 1995). Other reagents of the analytical grade came from the Polskie Odczynniki Chemiczne, Poland.

Ferredoxin:NADP oxidoreductase and ferredoxin were isolated from spinach purchased on the local market by the modified procedure described in Böger, Black, and San Pietro (1966) and Apley, Wagner, and Engelbrecht (1985) and their purities were confirmed by the Na-dodecyl sulphate polyacrylamide gel electrophoresis. The A_{495}/A_{276} ratio for FNR and the A_{420}/A_{276} ratio for Fd were 0.18 and 0.28, respectively. FNR and Fd concns were calculated using extinction coefficients 9.8 mM^{-1} cm^{-1} at 420 nm and 11.3 mM^{-1} cm^{-1} at 459 nm, respectively. For other details see Bojko and Wieckowski (1995).

The quinones were added to the reaction mixture as MeOH soln and their concn determined spectrophotometrically using molar absorption coefficients given in Kruk, Strzałka, and Leblanc (1992). The final conc. of MeOH in the reaction mixture did not exceed 2%.

The O_2 uptake was monitored at 25°C by a Clark-type O_2 electrode (Hansatech., UK) connected with the TZ 4100 Line Recorder (Praha, The Czech Republic).

The absorbance measurements were carried out by the LSM/Aminco DW 2000 Spectrophotometer (USA).

Acknowledgements

This study was financially supported by the Committee for Scientific Researches (KBN), grant No. 6P04A 049 08. The authors are grateful to Dr. Grażyna Majewska for assistance in the isolation of FNR and Fd.

References

Anusevicius, Z., Martinez-Julvez, M., Genzor, C. G., Nivinskas, H., Gomez-Moreno, C., & Cenas, N. (1997). *Biochimica et Biophysica Acta, 1320,* 247.

Apley, E. C., Wagner, R., & Engelbrecht, S. (1985). *Analytical Biochemistry, 150,* 145.

Arakaki, A. K., Ceccarelli, E. A., & Carrillo, N. (1997). *FASEB Journal, 11,* 133.

Batie, Ch. J., & Kamin, H. (1981). *Journal of Biological Chemistry, 256,* 7756.

Bendall, D. S., & Monasse, R. S. (1995). *Biochimica et Biophysica Acta, 1229,* 23.

Böger, P. (1971). *Zeitschrift für Naturforschung, 26b,* 807.

Böger, P., Black, C. C., & San Pietro, A. (1966). *Archives of Biochemistry and Biophysics, 115,* 35.

Bojko, M., & Wieckowski, S. (1995). *Phytochemistry, 40,* 661.

Bowyer, J. R., O'Neill, P., Camilleri, P., & Todd, C. M. (1988). *Biochimica et Biophysica Acta, 932,* 124.

Bruns, Ch. M., & Karplus, P. A. (1995). *Journal of Molecular Biology, 247,* 125.

Carrillo, N., & Vallejos, R. H. (1987). In J. Barber (Ed.), *Topics in photosynthesis* (Vol. 8, p. 527). Amsterdam, New York, Oxford: Elsevier.

Chang, K.-T., Morrow, K. J., Jr, Hirasawa, M., & Knaff, D. B. (1991). *Archives of Biochemistry and Biophysics, 290,* 522.

Forti, G., & Sturani, E. P. (1968). *European Journal of Biochemistry, 3,* 461.

Guedeney, G., Corneille, S., Cuine, S., & Peltier, G. (1995). In: P. Mathis, *Photosynthesis from light to biosphere* (Vol. 2, p. 883). Dordrecht, Boston, London: Kluwer Academic Publ.

Hosler, J. P., & Yocum, C. F. (1985). *Biochimica et Biophysica Acta, 808,* 21.

Kruk, J., Jemiola-Rzeminska, M., & Strzałka, K. (1997). *Chemistry and Physics of Lipids, 87,* 73.

Kruk, J., Strzałka, K., & Leblanc, R. M. (1992). *Biochimica et Biophysica Acta, 1112,* 19.

Lenaz, G., Espost, M. D., & Castelli, G. P. (1982). *Biochemical and Biophysical Research Communication, 105,* 589.

Morrison, L. E., Schelhorn, J. E., Cotton, T. M., Bering, Ch. L., & Loach, P. A. (1982). In B. L. Trumpower (Ed.), *Function of quinones in energy conserving systems* (p. 35). New York, London: Academic Press.

Moss, D. A., & Bendall, D. S. (1984). *Biochimica et Biophysica Acta, 767,* 389.

Nielsen, H. L., Andersen, B., & Scheller, H. V. (1995). In: P. Mathis, *Photosynthesis from light to biosphere* (Vol. 2, p. 847). Dordrecht, Boston, London: Kluwer Academic Publ.

Radunz, A., & Schmid, G. H. (1973). *Zeitschrift für Naturforschung, 28c,* 36.

Ravenel, J., Peltier, G., & Havaux, M. (1995). *Planta, 193,* 251.

Schmid, G. H., Radunz, A., Bader, K. P., Mysliwa-Kurdziel, B., Strzałka, K., & Kruk, J. (1996). *Naturforschung, 51c,* 691.

Shahak, Y., Crowther, D., & Hind, G. (1981). *Biochimica et Biophysica Acta, 636,* 234.

Sugioka, K., Nakana, M., Totsune-Nakano, H., Minokami, H. M., Kubata, T., & Ikegami, Y. (1988). *Biochimica et Biophysica Acta, 936,* 377.

Swallow, A. J. (1982). In: B. L. Trumpower, *Function of quinones in energy conserving systems* (p. 59). New York, London: Academic Press.

Wieckowski, S., & Bojko, M. (1997). *Photosynthetica, 34,* 481.

Zanetti, G. (1981). *Plant Science Letters, 23,* 55.

Zanetti, G., & Forti, G. (1966). *Journal of Biological Chemistry, 241,* 279.