Biochimica et Biophysica Acta, 680 (1982) 297–303
Elsevier Biomedical Press

BBA 41088

KINETICS OF SOME ELECTRON-TRANSFER REACTIONS IN BIOLOGICAL PHOTOSYSTEMS

II. STUDY OF INTRAMOLECULAR ELECTRON-TRANSFER RATES BETWEEN FERREDOXIN-NADP REDUCTASE, NADP· RADICAL AND OXIDIZED FERREDOXIN

RICHARD MASKIEWICZ and BENON H.J. BIELSKI *

Department of Chemistry, Brookhaven National Laboratory, Upton, NY 11973 (U.S.A.)

(Received October 21st, 1981)
(Revised manuscript received February 8th, 1982)

Key words: Electron-transfer rate; NADP· radical; Ferredoxin; Ferredoxin-NADP reductase

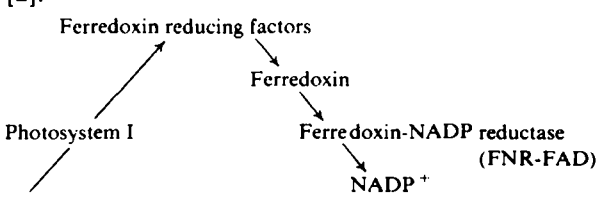
It has been shown by the pulse radiolysis technique that radiation-generated NADP free radicals (NADP·) first combine with ferredoxin-NADP reductase and then transfer the odd electron by a fast intramolecular process to the enzyme flavin moiety yielding the semiquinone (ferredoxin-NADP reductase, FNR-FADH·). The corresponding first-order rate constant k_{15} varies with ionic strength from $2.6 \cdot 10^3$ s^{-1} at $I = 0.66$ M to $2.3 \cdot 10^4$ s^{-1} at $I = 0.005$ M. In the presence of ferredoxin-NADP reductase-bound oxidized ferredoxin, the electron cascades, thus further reducing the ferredoxin. The transfer of the electron from the flavin semiquinone (ferredoxin-NADP reductase, FNR-FADH·) to the bound oxidized ferredoxin proceeds at a rate of $k_{18} = 2.36$ s^{-1}. This process approaches an equilibrium condition which is in favor of the reverse reaction suggesting that $k_{-18} > k_{18}$.

Introduction

Ferredoxin-NADP reductase (FNR-FAD) is a flavoprotein found in chloroplasts which catalyzes the reduction of $NADP^+$ by reduced ferredoxin (Fd_{red}) [1]:

$$2Fd_{red} + NADP^+ + H^+ \overset{(FNR\text{-}FAD)}{\rightleftharpoons} 2Fd_{ox} + NADPH \qquad (1)$$

The enzyme has been shown to occupy a position in the electron-transport chain past Photosystem I [2]:



Since the reductase is a flavoprotein it can exist in three redox states, fully oxidized (ferredoxin-NADP reductase, FNR-FAD), as a semiquinone (ferredoxin-NADP reductase, FNR-FADH·) or fully reduced (ferredoxin-NADP reductase, FNR-$FADH_2$) [3]. The multiple oxidation states of the flavin moiety allow the enzyme to couple overall two-electron redox processes in two one-electron step reactions known to proceed via the semiquinone radical [1,3], i.e.:

$$FNR\text{-}FAD + Fd_{red} \underset{-H^+}{\overset{+H^+}{\rightleftharpoons}} FNR\text{-}FADH\cdot + Fd_{ox} \qquad (2)$$

$$FNR\text{-}FADH\cdot + Fd_{red} \underset{-H^+}{\overset{+H^+}{\rightleftharpoons}} FNR\text{-}FADH_2 + Fd_{ox} \qquad (3)$$

Because of these properties it has been suggested that it may be at this point in the electron-transport path where the transition from one-electron to

* To whom correspondence should be addressed.

two-electron transport occurs [3].

Recent studies on ferredoxin-$NADP^+$ reductase have been primarily directed toward the elucidation of its structure [4,5], the location and environment of its binding sites [4–8], conformational changes upon complex formation [9], formation of complexes [10–17], the effect of ionic strength upon complex stability [18–20] and its role in cyclic electron transport [21,22]. The objective of the present study is the measurement of some of the intracomplex electron-transfer reactions.

Presuming that NADP redox reactions can occur in one-electron steps in this system, the semiquinone radical form of the enzyme may undergo the following reactions:

$$\text{FNR-FADH}^{\cdot} + \text{NADP}^+ \underset{+H^+}{\overset{H^+}{\rightleftharpoons}} \text{FNR-FAD} + \text{NADP}^{\cdot} \quad (4, -4)$$

$$\text{FNR-FADH}^{\cdot} + \text{NADP}^{\cdot} \rightleftharpoons \text{FNR-FAD} + \text{NADPH} \quad (5, -5)$$

Some of the steps in these electron-transfer reactions can be studied conveniently by the pulse radiolysis technique. This method affords a way to generate rapidly (micro- to nanoseconds) strong reducing or oxidizing radicals homogeneously distributed throughout the aqueous sample. By use of suitable scavengers, specific oxidizing or reducing species can be selectively generated from the primary radicals formed in reaction I. For example, the $NAD^{\cdot}$ [23,24] and $RF^{\cdot}$ (riboflavin) [25] radicals were shown to be formed in the following reactions:

$$H_2O \sim\sim OH(2.74), e_{aq}^-(2.76), H(0.55), H_2(0.45), H_2O_2(0.72)\ [26] \quad (I)$$

$$N_2O + e_{aq}^- + H_2O \rightarrow N_2 + OH^- + OH \quad k_6 = 8.7 \cdot 10^9\ s^{-1}\ [27] \quad (6)$$

$$HCOO^- + OH \rightarrow CO_2^- + H_2O \quad k_7 = 2.8 \cdot 10^9\ M^{-1} \cdot s^{-1}\ [28] \quad (7)$$

$$HCOO^- + H \rightarrow CO_2^- + H_2 \quad k_8 = 3.0 \cdot 10^8\ M^{-1} \cdot s^{-1}\ [29] \quad (8)$$

$$NAD^+ + CO_2^- \rightarrow NAD^{\cdot} + CO_2 \quad k_9 = 1.6 \cdot 10^9\ M^{-1} \cdot s^{-1}\ [23,24] \quad (9)$$

$$RF + CO_2^- \rightarrow RF^- + CO_2 \quad k_{10} = 3.6 \cdot 10^9\ M^{-1} \cdot s^{-1}\ [25] \quad (10)$$

where the numerical values in parentheses in Eqn. I are G values, i.e., the number of molecules formed or transformed per 100 eV of energy dissipated in the system.

Recent work on the reduction of FAD by $NAD^{\cdot}$ (Greenstock, E.L., private communication) has shown that the reaction proceeds at near diffusion-controlled rates:

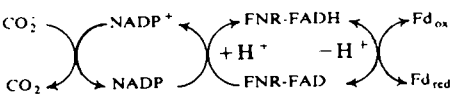
$$FAD + NAD^{\cdot} \rightarrow FADH^{\cdot} + NAD^+ \quad k_{11} = 1.2 \cdot 10^9\ M^{-1} \cdot s^{-1} \quad (11)$$

It has als been shown that $NAD^{\cdot}$ and $NADP^{\cdot}$ readily bind to enzymes employing nicotinamides as cofactors or substrates [24,30]. The corresponding dissociation constants for dehydrogenases range from 10^{-6} to 10^{-7} M [24].

Although the reduction of ferredoxin by CO_2^- had already been reported earlier [31], we found it necessary to reinvestigate the system in greater detail and under present experimental conditions. The results are reported in the first paper [32] of this series of studies. The reduction of ferredoxin by CO_2^- is a direct electron transfer to the iron-sulfur cluster of the protein:

$$Fd_{ox} + CO_2^- \rightarrow Fd_{red} + CO_2 \quad k_{12} = 6.2 \cdot 10^7\ M^{-1} \cdot s^{-1} \quad (12)$$

The present investigation was designed to study the electron transfer from $NADP^{\cdot}$ to the flavin moiety of ferredoxin-NADP reductase, and if possible from the enzyme semiquinone radical (ferredoxin-NADP reductase, FNR-FADH$^{\cdot}$) to oxidized ferredoxin. By the choice of proper concentrations of the various components of the system the electron should cascade as outlined in Eqn. II:



(II)

Experimental Procedure

Ferredoxin-NADP reductase was obtained from Sigma Chemical Co. (St. Louis, MO) and purified by the method of Shin [33]. Spinach ferredoxin was also obtained from Sigma Chemical Co., and was purified by the method described by Petering

and Palmer [34]. Before use in radiolysis experiments, Tris-HCl buffer (0.15–0.3 M), pH 7.5, was removed from protein samples by dialysis at 4°C against 0.01 M phosphate or argon-saturated water.

Sodium formate was prepared by three recrystallizations in the presence of EDTA and twice from water alone. The sodium salt of $NADP^+$ was used as supplied by Sigma Chemical Co. Solutions for use in pulse radiolysis were prepared by dissolving sodium formate and $NADP^+$ (sodium salt) in ultrapure water and degassing by bubbling with prepurified N_2O. Since ferredoxin-NADP reductase foams upon bubbling, the protein was degassed by lyophilization or by several freeze-thaw cycles. All experiments were performed anaerobically under N_2O or argon.

Pulse radiolysis experiments were carried out with a 2 MeV Van de Graaff generator, using 2–3-μs pulses. The data were analyzed using a PDP-11 computer interfaced to both the Van de Graaff and the photodetector. The number of free radicals formed per pulse was computed from ferrous dosimeter calibrations taking $G(Fe^{3+}) = 15.6$ [35].

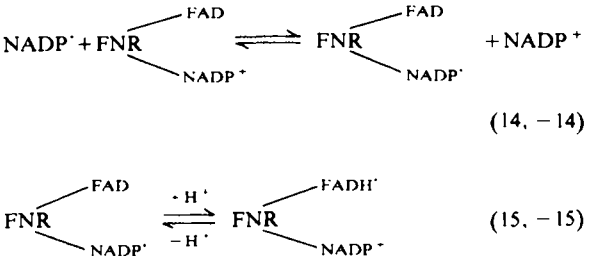
Results and Discussion

As a preliminary to studying the reduction of ferredoxin-NADP reductase by biologically relevant species (i.e., NADP$^\cdot$), the reduction of this enzyme by CO_2^- was examined. Reduction of the active-site flavin was carried out at pH 7.1 and 25.1°C under pseudo-first-order kinetics in N_2O-saturated 0.1 M formate containing the enzyme. The computed upper limit for the second-order rate constant $k_{13} = (3.0 \pm 0.3) \cdot 10^7\ M^{-1} \cdot s^{-1}$ should be compared with an earlier reported value for the reduction of riboflavin by CO_2^- ($k_{10} = 3.6 \cdot 10^9\ M^{-1} \cdot s^{-1}$) [25]:

$$\text{FNR-FAD} + CO_2^- \xrightarrow{H^+} \text{FNR-FADH}^\cdot + CO_2 \qquad (13)$$

The difference in rates for reactions 10 and 13 by two orders of magnitude suggests that the flavin moiety in ferredoxin-NADP reductase is not easily accessible to a nonspecific reactant considering that CO_2^- is a relatively small molecule.

The use of a natural substrate radical, i.e., NADP$^\cdot$ formed radiolytically in 0.1 M formate (Reactions 6–9), results in a rapid reduction of the oxidized flavin active site of the enzyme to the corresponding semiquinone state as shown in Fig. 1:



As is apparent from Fig. 1, the spectrum of ferredoxin-NADP reductase (FNR-FADH$^\cdot$) is very similar to those obtained upon radiolytic reduction of riboflavin [25], flavodoxin [36] and the NADPH reduction of ferredoxin-NADP reductase [1]. The transient spectrum of NADP$^\cdot$ with an observed maximum at 400 nm corresponds more closely to unbound NADP$^\cdot$ (λ_{max} 400 nm) [23,24] than to enzyme-bound NADP$^\cdot$ (i.e., malate dehydrogenase-NAD$^\cdot$ has a $\lambda_{max} \approx 425$ nm) [24]. Furthermore, no shift in wavelength of the peak at 400 nm was noticed with time, suggesting either a low steady-state concentration of bound NADP$^\cdot$ or a minimal spectral perturbation upon binding. The latter would be in agreement with the suggestion that binding of NADP to ferredoxin-NADP reductase is primarily electrostatic in nature [33,37].

Since in the present experiments [$NADP^+$]/[$NADP^+$-enzyme] is maintained at a high value, NADP$^\cdot$ is formed preferentially in the bulk of the solution and not on the enzyme surface. Once formed, NADP$^\cdot$ can either dimerize to the inactive form $(NADP)_2$ or react with the enzyme. A quantitative extrapolation at 400 nm to the end of the electron pulse indicates that initially 3.3 μM NADP$^\cdot$ was formed which yielded ultimately (0.94 ms after the pulse) 2.8 μM ferredoxin-NADP reductase (FNR-FADH$^\cdot$), observed at 540 and 555 nm. Unfortunately, in this system we were unable to distinguish spectrally between free and enzyme-bound NADP$^\cdot$ and hence could not determine the pathway (i.e., electron transfer or displacement of $NADP^+$ by NADP$^\cdot$) nor the rate

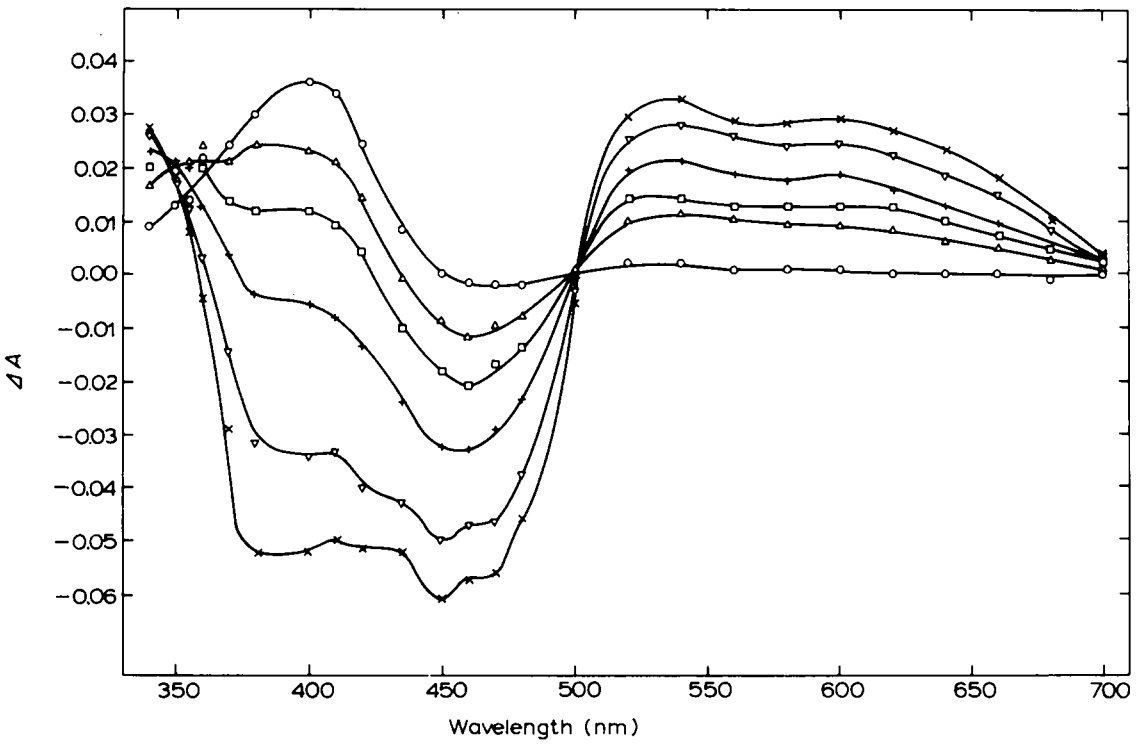


Fig. 1. Changes in absorption spectrum with time due to electron transfer from NADP$^{\cdot}$ to ferredoxin-NADP reductase following a 2 μs electron pulse: ○, 12 μs; △, 44 μs; □, 75 μs; +, 140 μs; ▽, 330 μs; ×, 940 μs. A 0.1 M formate solution, pH 7.1, and at 25.1°C contained 8.54 μM ferredoxin-NADP reductase and 1 mM $NADP^+$. The electron pulse produced 3.3 μM NADP$^{\cdot}$. The optical light path was 6.1 cm.

constant for reaction 14. In view of the high rate of NADP$^{\cdot}$ dimerization [23,24] ($k = 8 \cdot 10^7$ $M^{-1} \cdot s^{-1}$), the lower limit for k_{14} is approx. $2 \cdot 10^8$ $M^{-1} \cdot s^{-1}$, assuming that the lost NADP$^{\cdot}$ =

TABLE I

RATES OF REDUCTION OF FERREDOXIN-NADP REDUCTASE BY RADIATION-GENERATED NADP$^{\cdot}$ AS A FUNCTION OF WAVELENGTH

The reaction mixture contained after termination of a 2 μs electron pulse: 0.1 M formate, 1 mM $NADP^+$, 8.54 μM ferredoxin-NADP reductase and 1.1 μM NADP$^{\cdot}$ at $I = 0.1$ M, pH 7.1 and 25.1°C. Each rate value is an average of three experimental runs.

Wavelength (nm)	k_{15} (s^{-1}) ($\times 10^{-3}$)
380	4.75 ± 0.6
400	5.73 ± 0.6
460	6.22 ± 0.5
540	6.37 ± 0.5
600	5.59 ± 0.6

TABLE II

RATES OF FERREDOXIN-NADP REDUCTASE REDUCTION BY NADP$^{\cdot}$ AS A FUNCTION OF APPLIED DOSE

[ferredoxin-NADP reductase] = 8.5 μM; [$NADP^+$] = 1.0 mM; [HCOONa] = 0.1 M; pH 7.1 and 25.1°C; 10 rad = 0.6 μM CO_2^{-} or NADP$^{\cdot}$. Each value is an average of three runs. The average for all runs is $k_{15} = (5.53 \pm 0.7) \cdot 10^3$ s^{-1} at $I = 0.1$ M.

Dose (rad)	Wavelength (nm)	k_{15} (s^{-1}) ($\times 10^{-3}$)
7	410	6.05 ± 0.5
	460	5.85 ± 0.4
	540	4.80 ± 0.4
12	410	5.65 ± 0.5
	460	6.50 ± 0.5
	540	5.70 ± 0.4
20	410	4.75 ± 0.5
	460	6.60 ± 0.4
	540	4.55 ± 0.4
35	410	4.75 ± 0.4
	460	5.60 ± 0.5
	540	5.50 ± 0.5

($[NADP^{\cdot}]_o - [FNR\text{-}FADH^{\cdot}]_f$ is due to dimerization and not to formation of a very small fraction of totally reduced ferredoxin-NADP reductase ($FNR\text{-}FADH_2$).

The rate of reduction of the flavin moiety in ferredoxin-NADP reductase (monitored at 460 nm) equals the rate of $NADP^{\cdot}$ disappearance (400 nm) and ferredoxin-NADP reductase semiquinone formation ($FNR\text{-}FADH^{\cdot}$) observed at 540 nm. As shown in Table I, the rates for k_{15} measured at these selected wavelengths are ($k_{15} = 5.7 \pm 0.6 \cdot 10^3$ s^{-1} at $I = 0.1$ M), within experimental error, similar.

Dose-rate experiments (Table II), i.e., studies of the effect of $NADP^{\cdot}$ concentration upon the rate of ferredoxin-NADP reductase semiquinone ($FNR\text{-}FADH^{\cdot}$) generation, show that the observed first-order rate constant is independent of the quantity of $NADP^{\cdot}$ added at constant ferredoxin-NADP reductase concentration. Also, a study of the rate of reduction of ferredoxin-NADP reductase by $NADP^{\cdot}$ (0.51 μM) over a 10-fold enzyme concentration range (3.8–39.0 μM) yielded first-order rate constants which were independent of the enzyme concentration and within experimental error similar to the values reported in Tables I and II.

As illustrated in Fig. 2, the value of k_{15} varies

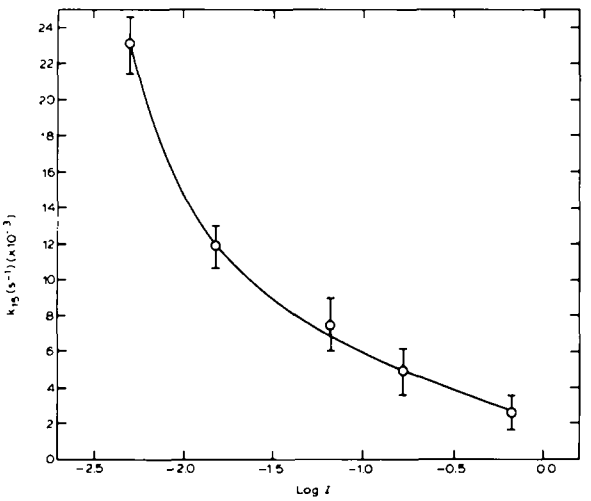


Fig. 2. Rates of ferredoxin-NADP reductase reduction by $NADP^{\cdot}$ as a function of ionic strength; [ferredoxin-NADP reductase] = 9.1 μM; $[NADP^+]$ = 0.5 mM; [HCOONa] = 0.05–0.665 M; $[NADP^{\cdot}]$/pulse = 1.1 μM; pH 6.8–7.1, 25°C. Rates were monitored at 540 nm.

with ionic strength, which was adjusted by the addition of sodium formate. In going from an ionic strength of $I = 0.66$ M ($k_{15} = 2.6 \cdot 10^3$ s^{-1}) to $I = 0.005$ M ($k_{15} = 2.3 \cdot 10^4$ s^{-1}), the average value of k_{15} increased by one order of magnitude, suggesting conformational changes with change in ionic strength.

The fact that $NADP^{\cdot}$ reduces ferredoxin-NADP reductase much more rapidly than does CO_2^- suggests that the reaction of $NADP^{\cdot}$ and ferredoxin-NADP reductase involves more than a simple electron transfer. In view of the kinetic evidence we propose that the observed reaction 15 represents an intramolecular electron transfer from bound $NADP^{\cdot}$ to the enzyme FAD moiety, the binding of $NADP^{\cdot}$ or its electron transfer to the enzyme-NAD^+ (reaction 14) being very rapid. While binding of $NADP^{\cdot}$ to ferredoxin-NADP reductase most likely stabilizes the free radical thus promoting reaction 15, it also prevents or slows down the dimerization process [24,30].

Subsequent to the reduction of ferredoxin-NADP reductase by $NADP^{\cdot}$, a slow reaction with an approximate half-life of $t_{1/2} = 1.63$ s ($\pm 10\%$) was observed which resulted in a decrease in absorbance at 540 nm (ferredoxin-NADP reductase semiquinone, $FNR\text{-}FADH^{\cdot}$) and an increase in absorbance at 460 nm (oxidized ferredoxin-NADP reductase, FNR-FAD). Although the observed absorbance changes did not follow any specific kinetics over the time period of the measurement, the half-lives at the two wavelengths were similar, suggesting a slow disproportionation process to oxidized (ferredoxin-NADP reductase, FNR-FAD) and reduced (ferredoxin-NADP reductase, $FNR\text{-}FADH_2$) enzyme.

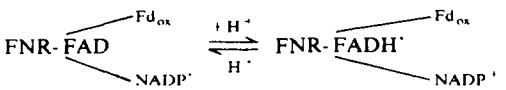
When the reduction of ferredoxin-NADP reductase by $NADP^{\cdot}$ is carried out in the presence of oxidized spinach ferredoxin and under conditions where the electron can cascade to the ferredoxin, an increase in the rate of ferredoxin-NADP reductase semiquinone ($FNR\text{-}FADH^{\cdot}$) decay (now strictly first order) is observed:

$$CO_2^- + NADP^+ \rightarrow CO_2 + NADP^{\cdot} \quad k_9 = 1.6 \cdot 10^9\ M^{-1} \cdot s^{-1} \quad (9)$$

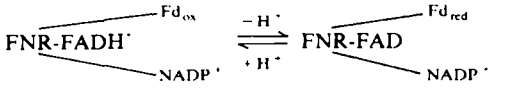
$$NADP^{\cdot} + FNR\text{-}FAD \underset{NADP^+}{\overset{Fd_{ox}}{\rightleftharpoons}} FNR\text{-}FAD \underset{NADP^{\cdot}}{\overset{Fd_{ox}}{}}$$

$$+ NADP^+ \quad k_{16} \text{ assumed fast}$$

(16, −16)



$k_{17} = 3.3 \cdot 10^4\ s^{-1}$ (17, −17)



$k_{18} = 2.36\ s^{-1}$ (18, −18)

This system was studied at two enzyme and ferredoxin concentrations, in order to verify that the observed rates for reactions 17 and 18 are independent of the concentrations of these components: $[\text{FNR-FAD}]_1 = 13.3\ \mu M$ and $[Fd_{ox}]_1 = 3.5\ \mu M$; $[\text{FNR-FAD}]_2 = 34.4\ \mu M$ and $[Fd_{ox}]_2 = 8.6\ \mu M$; $[NADP^+] = 200\ \mu M$; $[NADP^\cdot] = 0.9\ \mu M$/pulse; $I = 0.005$ M; 23.5°C and pH 6.9. The measured rates for reaction 17 gave an average value for $k_{17} = (3.3 \pm 0.3) \cdot 10^4\ s^{-1}$ which is approx. 43% higher than the corresponding value for reaction 15 at $I = 0.005$ M in the absence of oxidized ferredoxin. Whether this small increase is of any significance is difficult to tell; it could be due to either a conformational change or the close proximity of the ferredoxin iron-sulfur cluster.

In monitoring absorbance changes at 500 nm where flavoprotein absorbance changes show an isosbestic point but where ferredoxin absorbance decreases upon reduction, a first-order reaction was observed for reaction 18, $k_{18} = 2.36 \pm 0.63\ s^{-1}$. This rate constant measures an intramolecular electron transfer as depicted in Eqn. 18, since under our experimental conditions, i.e., at $I = 0.005$ M, the added oxidized ferredoxin was essentially bound quantitatively to ferredoxin-NADP reductase if we accept that the dissociation constant for the enzyme-ferredoxin complex is $K_d = 1.53 \cdot 10^{-7}$ M [37]. Similar rates for k_{18} were measured at 540 nm for the disappearance of ferredoxin-NADP reductase semiquinone (FNR-FADH$^\cdot$) and formation of ferredoxin-NADP reductase (FNR-FAD) at 460 nm.

As is apparent from the large error in k_{18}, this value must be treated with caution as it most likely includes a contribution (nonresolvable) from the spontaneous decay of ferredoxin-NADP reductase semiquinone (FNR-FADH$^\cdot$) observed in the absence of ferredoxin. Whether the spontaneous decay of ferredoxin-NADP reductase semiquinone (FNR-FADH$^\cdot$) is accelerated by ferredoxin is unknown. A comparison of total absorbance changes at 460 and 540 nm shows good agreement on a molar basis for the disappearance of ferredoxin-NADP reductase semiquinone (FNR-FADH$^\cdot$) and the appearance of oxidized enzyme (FNR-FAD). Inspection of the total absorbance change at 500 nm (isosbestic point for the flavin system) shows that only 10–15% ferredoxin reduction occurred taking the amount of ferredoxin-NADP reductase semiquinone (FNR-FADH$^\cdot$) formed as 100%. Overall, the system appears to reach an equilibrium situation with respect to ferredoxin, suggesting that k_{-18} is larger than k_{18} in agreement with the generally observed direction of electron flow when an excess of reduced ferredoxin is added to this enzyme system.

Acknowledgement

This research was carried out at Brookhaven National Laboratory under contract with the U.S. Department of Energy and supported by its Division of Chemical Sciences, Office of Basic Energy Sciences.

References

1 Shin, M. and Arnon, D.I. (1965) J. Biol. Chem. 240, 1405–1411
2 Avron, M. (1975) in Bioenergetics of Photosynthesis (Govindjee, ed.), p. 376, Academic Press, New York London
3 Forti, G., Melandri, B.A., San Pietro, A. and Ke, B. (1970) Arch. Biochem. Biophys. 140, 107–112
4 Ellefson, W.L. and Krogmann, D.W. (1979) Arch. Biochem. Biophys. 194, 593–599
5 Sheriff, S. and Herriot, J.R. (1981) J. Mol. Biol. 81, 441–451
6 Davis, D.J. and San Pietro, A. (1977) Arch. Biochem. Biophys. 184, 572–577
7 Zanetti, G., Gozzer, C., Sacchi, G. and Curti, B. (1979) Biochim. Biophys. Acta 568, 127–134
8 Carrillo, N., Arana, J.L. and Vallejos, R.H. (1981) J. Biol. Chem. 256, 6828–6823
9 Wagner, R., Carrillo, N., Junge, W. and Vallejos, R. (1981) FEBS Lett. 131, 335–340
10 Nelson, N. and Neumann, J. (1969) J. Biol. Chem. 244, 1932–1936
11 Shin, M. (1973) Biochim. Biophys. Acta 292, 13–19
12 Davis, D.J. and San Pietro, A. (1977) Biochem. Biophys. Res. Commun. 74, 33–40

13 Khristin, M.S. and Akulova, E.A. (1978) Dokl. Akad. Nauk S.S.S.R. 238, 992–995
14 Bookjans, G. and Boeger, P. (1979) Arch. Biochem. Biophys. 194, 387–393
15 Ricard, J., Nari, J. and Diamantidis, G. (1980) Eur. J. Biochem. 108, 55–66
16 Knaff, D.B., Smith, J.M. and Chain, R.K. (1980) Arch. Biochem. Biophys. 199, 117–122
17 Zanetti, G. and Curti, B. (1981) FEBS Lett. 129, 201–204
18 Nakamura, S. and Kimura, T. (1971) FEBS Lett. 15, 353–354
19 Batie, C.J. and Kamin, H. (1981) J. Biol. Chem. 256, 7756–7763
20 Hasumi, H., Nakamura, S., Koga, K. and Yoshizumi, H. (1979) Biochem. Biophys. Res. Commun. 87, 1095–1101
21 Boehme, H. (1977) Eur. J. Biochem. 72, 283–289
22 Shahak, Y., Crowther, D. and Hind, G. (1981) Biochim. Biophys. Acta 636, 234–243
23 Land, E.J. and Swallow, A.J. (1968) Biochim. Biophys. Acta 162, 327–337
24 Bielski, B.H.J. and Chan, P.C. (1980) J. Am. Chem. Soc. 102, 1713–1716
25 Land, E.J. and Swallow, A.J. (1969) Biochemistry 8, 2117–2125
26 Bielski, B.H.J. and Allen, A.O. (1969) Int. J. Radiat. Phys. Chem. 1, 153–163
27 Gordon, S., Hart, E.J., Matheson, M.S., Rabani, J. and Thomas, K. (1963) Disc. Faraday Soc. 36, 193–205
28 Willson, R.L., Greenstock, C.L., Adams, G.E., Wageman, R. and Dorfman, L.M. (1971) 3, 311–220
29 Burchill, C.E., Dainton, F.S. and Smithies, D. (1967) Trans. Faraday Soc. 63, 932–943
30 Chan, S.S., Norlund, T.M., Frauenfelder, H., Harrison, J.E. and Gunsalus, I.C. (1975) J. Biol. Chem. 250, 716–719
31 Pecht, I. (1973) FEBS Lett. 33, 259–262
32 Maskiewicz, R. and Bielski, B.H.J. (1981) Biochim. Biophys. Acta 638, 153–160
33 Shin, M. (1970) Methods Enzymol. 23, 440–447
34 Petering, D.H. and Palmer, G. (1970) Arch. Biochem. Biophys. 141, 456–464
35 Allen, A.O. (1961) in The Radiation Chemistry of Water and Aqueous Solutions, p. 35, Van Nostrand, Princeton, NJ
36 Faraggi, M. and Klapper, M.H. (1979), J. Biol. Chem. 254, 8139–8142
37 Foust, G.P., Mayhew, S.G. and Massey, V. (1969) J. Biol. Chem. 244, 964–970