FERREDOXINS AND FERREDOXIN-NADP$^+$ REDUCTASES FROM ANABAENA PCC 7119 AND SPINACH: ELECTROSTATIC EFFECTS ON INTRACOMPLEX ELECTRON TRANSFER

M.C. Walker, J.A. Navarro[§], J.J Pueyo[#], C. Gómez-Moreno[#] and G. Tollin

Department of Biochemistry, University of Arizona, Tucson, AZ 85721, U.S.A. and [#]Departamento de Bioquímica y Biología Molecular y Celular, Universidad de Zaragoza, 50009 Zaragoza, Spain.

[§]Instituto de Bioquímica Vegetal y Fotosíntesis, Sevilla, Spain.

Introduction

The 1:1 complex of ferredoxin (Fd) and ferredoxin-NADP$^+$ reductase (FNR) at low ionic strength has been a model for redox interactions within electrostatically stabilized complexes (1-3). However, electron transfer between the proteins from spinach occurs too rapidly for stopped-flow experiments at low ionic strengths (1,2). Using laser flash photolysis, the first-order rate constant for intramolecular electron transfer within a transient complex between spinach Fd_{red} and FNR_{ox} at high ionic strengths (>310 mM) has been directly measured (3). This rate constant could not be determined at lower ionic strengths, since the Fe/S center of Fd_{ox} became inaccessible to direct reduction by deazariboflavin semiquinone (dRfH$^\cdot$) as a result of complex formation (3). This is not the case for the proteins from Anabaena PCC 7119 (A.), and we report here laser flash photolysis measurements of intracomplex electron transfer over a wider ionic strength range.

Results

Reduction of Anabaena Fd and FNR by dRfH$^\cdot$

A second-order rate constant for Fd reduction of 1.6 +/- 0.15 x 10^8 $M^{-1}s^{-1}$ was obtained at 10 mM ionic strength; at 310 mM ionic strength the rate constant was slightly increased (2.1 +/- 0.3 x 10^8 $M^{-1}s^{-1}$). These results are similar to those observed earlier for spinach Fd (3), although the rate constant increase at high ionic strength was much larger for spinach Fd. A second-order rate constant for FNR reduction of 4.2 +/- 0.2 x 10^8 $M^{-1}s^{-1}$ was obtained at an ionic strength of 10 mM; at 310 mM the rate constant was 3.2 x 10^8 $M^{-1}s^{-1}$. These results are again consistent with those obtained with the spinach protein (3).

Reduction of Anabaena FNR by Fd

Little or no direct reduction of A. FNR by dRfH$^\cdot$ was observed at I = 10 mM in the presence of

Flavins and Flavoproteins 1990

equimolar or greater amounts of A. Fd at FNR concentrations of up to 30 μM. This indicates that the accessibility of the FAD cofactor of FNR to dRfH is decreased when Fd is present at low ionic strength, presumably as a result of complex formation. Little or no decrease in Fd accessibility was noted. In contrast, the accessibility of the FAD of spinach FNR was found to be unaltered in the presence of spinach Fd at low ionic strengths, whereas the accessibility of the Fe/S center of the Fd was greatly diminished (3). The apparent inaccessibility of A. FNR to dRfH in the presence of Fd was unaffected by increases in ionic strength up to 310 mM. This suggests that electrostatic forces do not contribute significantly to the stability of the Fd_{ox}:FNR_{ox} complex, again in contrast to the spinach proteins (3,4,5). We conclude that the complexes between the oxidized proteins from A. and from spinach are structurally dissimilar and stabilized differently.

The observed rate constants for A. FNR reduction by Fd_{red} depended non-linearly on FNR concentration (Figure 1). This is consistent with a two-step mechanism in which the first-order

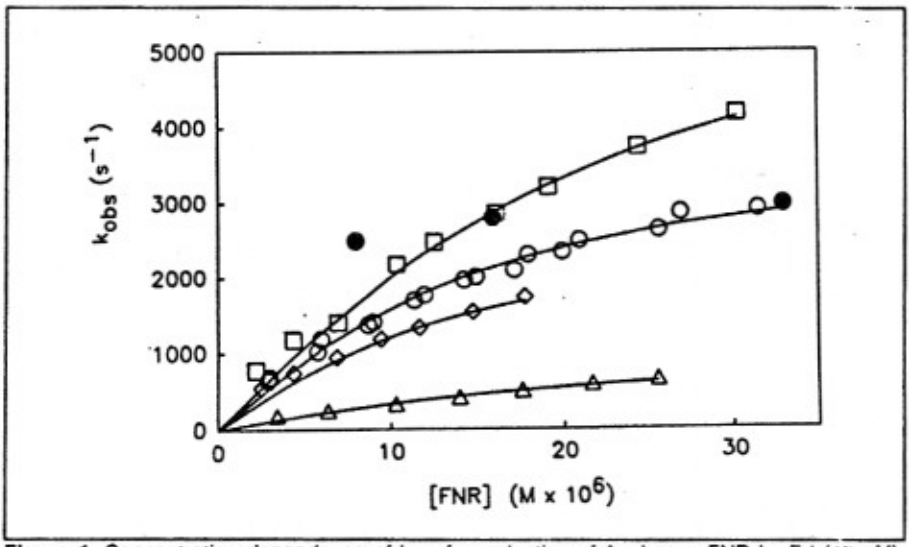

Figure 1: Concentration dependence of k_{obs} for reduction of Anabaena FNR by Fd (40 μM). Ionic strengths: (○) 10 mM; (□) 110 mM; (◇) 210 mM; (△) 310 mM.

reaction (k_{et}) becomes rate-limiting at high concentration:

$$Fd_{red} + FNR_{ox} \overset{K_d}{\rightleftharpoons} Fd_{red}{:}FNR_{ox} \xrightarrow{k_{et}} Fd_{ox}{:}FNR_{red}$$

The constants obtained from a computer fit of the data in Figure 1 to the above scheme (cf. 6 for details) are given in Table 1. The apparent K_d's are most likely influenced by the dissociation of the Fd_{ox}:FNR_{ox} complex, and thus represent upper limits. The constant was insensitive to ionic

strength (Table I), which again suggests that the stability of the oxidized complex is not dependent on electrostatic forces.

If the limiting first-order rate constant reflects intramolecular electron transfer within a 1:1 complex between Fd_{red}:FNR_{ox}, then its value should be independent of the concentration of the preformed complex. In order to confirm this, solutions of increasing equimolar concentrations of the two A. proteins at 10 mM ionic strength were subjected to laser photolysis (solid circles in Figure 1). The results are in agreement with expectation. The limiting rate constant varied with ionic strength, increasing to 6500 s^{-1} at 110 mM, then decreasing as the ionic strength was raised to 310 mM (Table I).

Table I: Kinetic Constants for Electron Transfer Within the Fd_{red}:FNR_{ox} Complexes from Anabaena PCC 7119 and Spinach.

Ionic Strength (mM)	K_d (x10^{-6} M)	k_{et} (s^{-1})
	Anabaena	
10	7.9	3830
110	12.1	6500
210	8	2800
310	10.5	1220
	Spinach	
110	2.5	3000
210	3.0	3300
310	10.7	2490
460	40.7	2240

Reduction of spinach FNR by Fd

The observed rate constant for the reduction of FNR (at constant Fd_{ox} concentration) at ionic strengths higher than 100 mM also displayed saturation kinetics as a function of FNR concentration (Figure 2). Unlike the A. proteins, the apparent K_d for the spinach Fd_{red}:FNR_{ox} complex at high ionic strengths is not significantly complicated by the formation of the fully oxidized complex. The constants from the computer analysis of the data in Figure 2 are included in Table I. The K_d value was found to increase substantially as the ionic strength was raised from 210 mM to 460 mM (Table I). These changes demonstrate the importance of electrostatic forces in the formation of the spinach Fd_{red}:FNR_{ox} complex.

Comparison of the results obtained at 210 mM and 110 mM indicate that there was little change in apparent K_d. Such a result is unexpected for an electrostatically stabilized complex. We ascribe this to increased competition from the formation of the Fd_{ox}:FNR_{ox} complex.

The limiting rate constant for intramolecular electron transfer from Fd_{red} to FNR_{ox} (k_{et}) was not appreciably affected by changes in ionic strength (cf. Table I). The increase reported earlier (3) upon changing the ionic strength from 310 mM to 460 mM was found to be due to a specific

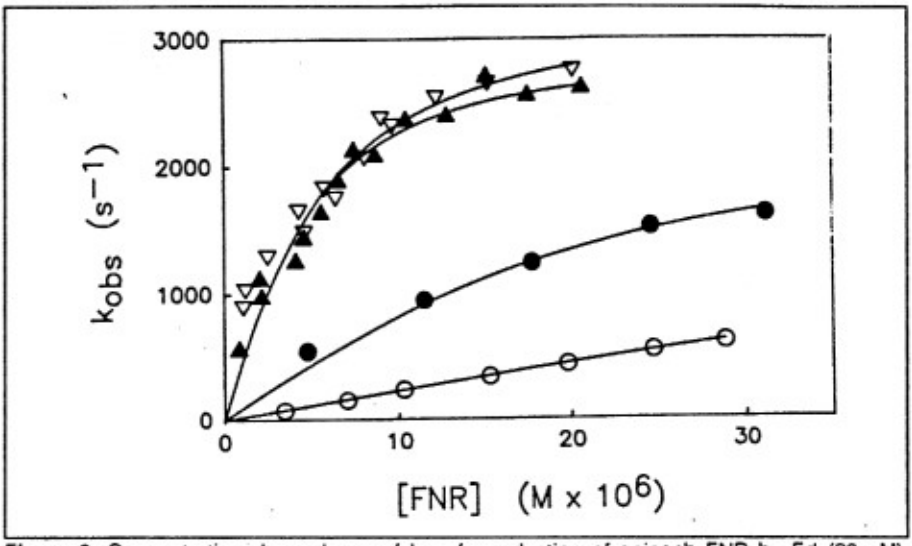

Figure 2: Concentration dependence of k_{obs} for reduction of spinach FNR by Fd (20 μM). Ionic strengths: (▲) 110 mM; (▽) 210 mM; (●) 310 mM; (○) 460 mM.

phosphate ion effect. The ionic strength independence of k_{et} suggests that electrostatic forces do not influence the electron transfer reaction between Fd_{red} and FNR_{ox} once the collision complex is formed, again in contrast with Anabaena.

Discussion

The limiting rate constant for electron transfer within the A. Fd_{red}:FNR_{ox} complex had a biphasic dependence on ionic strength. Thus, electrostatic forces probably assist in favorably orienting the two A. proteins during formation of the initial collision complex at ionic strengths from 310 to 110 mM, without providing a major contribution to complex stability. The decrease in rate constant below 110 mM implies that an optimal orientation for electron transfer is only achieved by additional protein rearrangement within the collision complex. This is inhibited by the stronger electrostatic interactions at 10 mM ionic strength, i.e. the proteins are "locked" into a less productive orientation. The absence of strong ionic strength effects on the kinetics of reduction of the free proteins by dRfH· makes unlikely the occurrence of conformational changes within the proteins which influence reactivity. With the spinach proteins, an increase in ionic strength had little effect on the limiting rate constant. This suggests that the relative orientation of the proteins must already be optimized for electron transfer within the initial collision complex.

Acknowledgement

Research supported by NIH Grant DK15057 (to G. T.) and Grant 0792/84 from Comision Asesora de Investigation Cientifica y Tecnica (to C. G.-M.).

References

1. Masaki, R., M. Matsumoto, S. Yoshikawa, H. Matsubara. 1982. In: Flavins and Flavoproteins (Massey, V. and C. H. Williams, eds.). Elsevier, New York. p. 675.

2. Batie, C. J., H. Kamin. 1984. J. Biol. Chem. 259, 11976.

3. Bhattacharyya, A. K., T. E. Meyer, G. Tollin. 1986. Biochemistry 25, 4655.

4. Foust, G. P., S. G. Mayhew, V. Massey. 1969. J. Biol. Chem. 244, 964.

5. Batie, C. J., H. Kamin. 1981. J. Biol. Chem. 256, 7756.

6. Simondsen, R. P., P. C. Weber, F. R. Salemme, G. Tollin. 1982. Biochemistry 21, 6366.