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A laser flash absorption spectroscopy study of *Anabaena* sp. PCC 7119 flavodoxin photoreduction by photosystem I particles from spinach

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Electron transfer from P700 in photosystem I (PSI) particles from spinach to *Anabaena* sp. PCC 7119 flavodoxin has been studied using laser flash absorption spectroscopy. A non-linear protein concentration dependence of the rate constants was obtained, suggesting a two-step mechanism involving complex formation (k=3.6 × 10^7 $M^{-1}·s^{-1}$) followed by intracomplex electron transfer (k=270 s^{-1}). The observed rate constants had a biphasic dependence on the concentrations of NaCl or $MgCl_2$, with maximum values in the 40–80 mM range for NaCl and 4–12 mM for $MgCl_2$. To our knowledge, this is the first time that the kinetics of PSI-dependent flavodoxin photoreduction have been determined.

Flavodoxin; Photosystem I; Electron transfer

1. INTRODUCTION

Flavodoxins (Fld) are low molecular weight flavin mononucleotide (FMN)-containing flavoproteins found in microorganisms and certain algae [1,2]. They can be synthesized either constitutively or induced by a lack of iron in the medium. In the latter case, Fld replaces ferredoxin (Fd) in all the reactions in which Fd participates [1]. Fld has been implicated in electron transfer from photosystem I (PSI) to the flavoprotein ferredoxin–NADP$^+$ reductase (FNR) [3,4]. The strain PCC 7119 of *Anabaena* induces the synthesis of large amounts of Fld under low iron conditions. The Fld has been purified and its properties [4,5] shown to be similar to those of other flavodoxins [1].

As is the case for Fd, most studies of the reduction of Fld by PSI have been carried out using indirect steady-state methods. We have recently reported kinetic measurements of electron transfer from spinach photosystem I (PSI) particles to spinach and algal Fd using laser flash absorption spectroscopy [6]. The present study complements this work by analogous measurements using spinach PSI and *Anabaena* Fld, thus permitting a comparison of the respective rate constants and the factors which control the reduction of both proteins. Such information can contribute to our understanding of why eukaryotes and some cyanobacteria have evolved so as to utilize Fd as the only PSI acceptor. To the best of our knowledge, this is the first report of a direct measurement of the rate constant for PSI-dependent Fld photoreduction.

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Abbreviations: DCPIP, dichlorophenolindophenol; Fld, flavodoxin; Fd, ferredoxin; FMN, flavin mononucleotide; FNR, ferredoxin-NADP$^+$ reductase; PSI, photosystem I; TSF-1, Triton-solubilized PSI particles; k_{obs}, observed rate constants.

2. MATERIALS AND METHODS

Triton-solubilized PSI particles (TSF-1) were isolated from spinach chloroplasts by the method of Vernon and Shaw [7], and resuspended in 10 mM HEPES-NaOH buffer, pH 7.0. The P700 content and chlorophyll concentration of the samples were obtained as previously described [6,8,9]. *Anabaena* sp. PCC 7119 Fld was purified and its concentration determined as reported earlier [4]. Unless otherwise noted, the standard reaction mixture contained, in a final volume of 0.2 ml, 10 mM HEPES buffer, pH 7.0, TSF-1 particles equivalent to 0.22 mg chlorophyll per ml, 20 μM DCPIP, 1 mM sodium ascorbate, 20 mM NaCl and 10 μM Fld.

The laser flash photolysis system [6,10,11] utilized a nitrogen laser-pumped dye solution (wavelength 660 nm) in a 4 cm pathlength rectangular cuvette. All experiments were carried out in a 2 mm pathlength cuvette at room temperature (23 ± 1°C) and each transient corresponded to the average of 10–20 laser flashes. The Fld concentration (>2 μM) was in excess over P700$^+$ so that pseudo-first order conditions existed. For most experiments, the error in the k_{obs} values for Fld reduction was estimated to be ≤±10%, based on reproducibility and on signal/noise ratios. For experiments carried out at extremes of pH or ionic strength, errors may be as large as ±20%.

3. RESULTS AND DISCUSSION

When PSI-enriched TSF-1 particles, supplemented with ascorbate and DCPIP to keep P700 reduced, are excited by a laser flash in the presence of Fld, P700 is

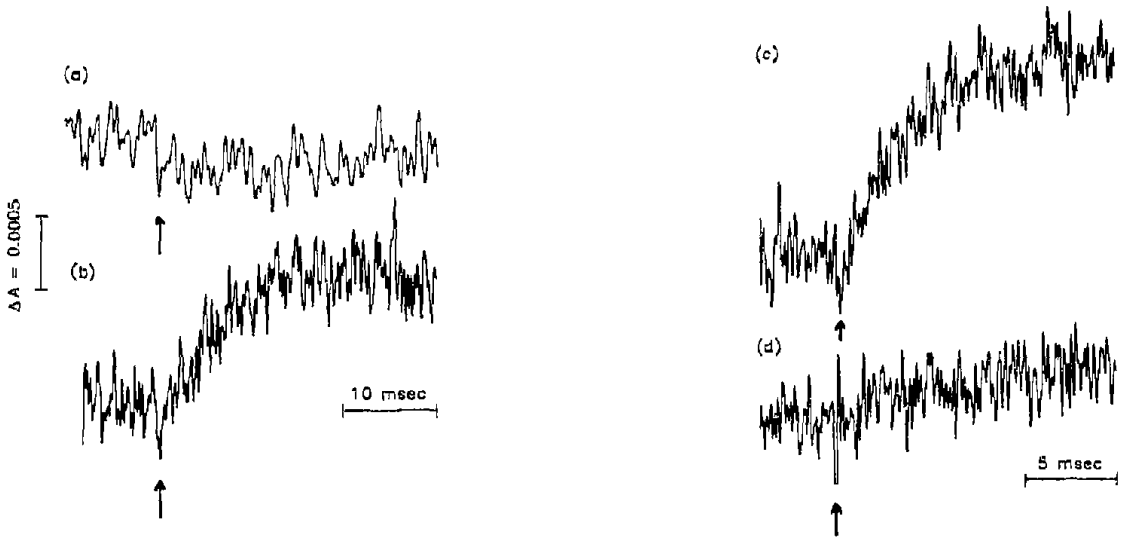

Fig. 1. Transient kinetics showing flash-induced electron transfer from P700 to Fld as measured by the absorbance changes at 575 nm. Reaction mixtures were as described in Materials and Methods, except that the Fld concentration was 0 (a), 4 μM (b) or 30 μM (c). The reaction mixture in (d) was as in (c) but supplemented with 0.1 mM methyl viologen. In each case, the arrow indicates the time at which the laser flash was triggered.

photooxidized [12] and Fld photoreduction occurs. The latter can be monitored by observing the formation of the FMN semiquinone, most conveniently at 575 nm [13] since at this wavelength PSI has an isosbestic point [12]. Curve a in Fig. 1 confirms that in the absence of added Fld no absorbance changes occur at 575 nm after excitation of PSI by the laser flash. In the presence of Fld, excitation induces an exponential increase in absorbance at 575 nm due to FMN semiquinone formation (curve b). This increase is enhanced in both amplitude and rate by addition of increasing amounts of Fld (curves b and c). In the presence of 0.1 mM methyl viologen (an effective electron acceptor from PSI) the absorbance changes at 575 nm are eliminated (curve d). Fld reduction is further confirmed by the absorbance decrease observed in the 470–480 nm region (data not shown), as expected from the reduced minus oxidized difference spectrum of Fld [13].

When the k_{obs} values measured at 575 nm are determined at various Fld concentrations, a non-linear dependence is obtained (Fig. 2). This implies a mechanism involving at least two steps, i.e. complex formation followed by intracomplex electron transfer [14]. Using a non-linear least squares fitting procedure [15], a value of 270 s^{-1} was obtained for the limiting intracomplex electron transfer rate constant. This is slightly larger than the corresponding rate constants for the PSI-dependent reduction of Fd from spinach (140 s^{-1}) and *Monoraphidium braunii* (180 s^{-1}) [6]. A value of 3.6×10^7 $M^{-1} \cdot s^{-1}$ for the second-order complex formation rate constant was also obtained from this analysis. This again is slightly larger than those for spinach (3.0×10^7 $M^{-1} \cdot s^{-1}$) and *M. braunii* (3.3×10^7 $M^{-1} \cdot s^{-1}$) Fd reduction by spinach PSI [6]. From the rate constant values one can infer that *Anabaena* Fld is slightly more reactive towards spinach PSI than spinach and *M. braunii* Fd with respect to both complex formation and intracomplex electron transfer, although the difference is more significant for the latter process. This may be a consequence of the redox potentials of these proteins (−0.42 V for Fd; −0.195 V for Fld [5]).

The effect of increasing salt concentrations on the kinetics is shown in Fig. 3a. The k_{obs} values increase with increasing NaCl concentration, having a maximum value at 40–80 mM NaCl, and decreasing again at higher salt concentrations. Similar biphasic dependencies have been found for the reduction of Fd by PSI [6], for the reduction of $P700^+$ by plastocyanin and cyto-

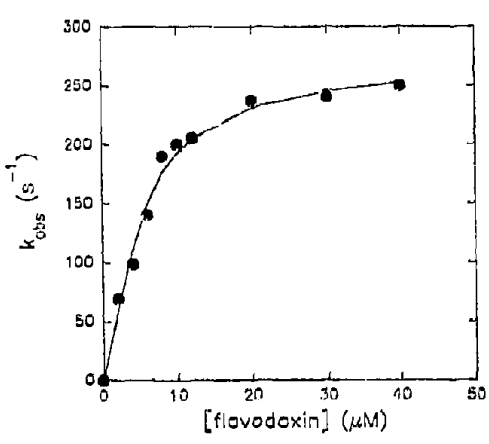

Fig. 2. Dependence of the k_{obs} values for Fld photoreduction on the concentration of added protein. Reaction mixtures were as described in Materials and Methods, but contained Fld at the indicated concentrations. The solid line corresponds to a theoretical fit to a two-step mechanism (see text).

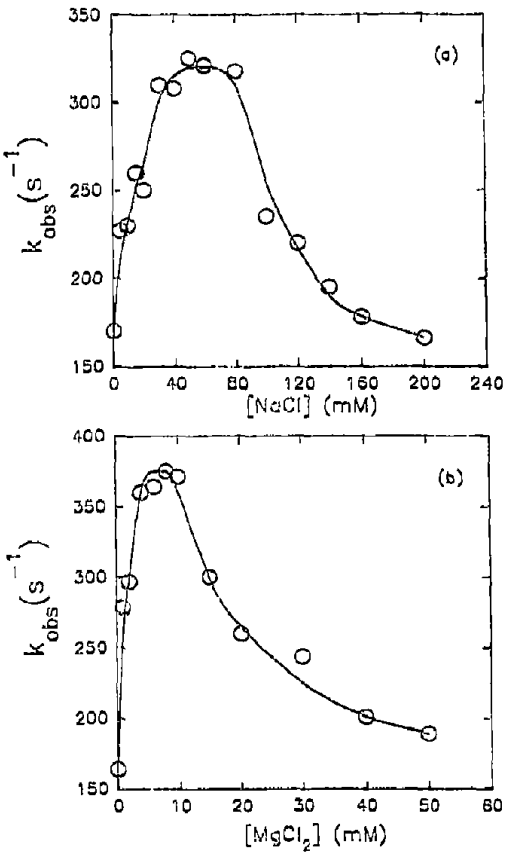

Fig. 3. Effect of NaCl (a) and $MgCl_2$ (b) on the rate constants for Fld reduction by PSI. Reaction mixtures were as described in Materials and Methods.

chrome c_6 [16], and for reactions between several non-photosynthetic redox proteins [17,18]. The interpretation of this behaviour is that an optimal orientation for electron transfer between the two oppositely charged reactants is achieved by an additional rearrangement occurring within an initially formed collision complex. This is inhibited by the stronger electrostatic forces at low ionic strengths. The decrease in rate constant at high ionic strengths is due to an increased probability of non-productive orientations resulting from weakening of the electrostatic forces, which serve to orientate the two proteins into an approximately correct configuration during the initial collision. It is not surprising that Fld behaves similarly to Fd [6], since the basic peptide that is purportedly involved in the interaction between Fd and PSI [19] probably also functions with Fld. Fig. 3b shows that the rate constant for Fld reduction by PSI also increases with increasing concentrations of $MgCl_2$, reaching a maximum value at 4–12 mM, and decreasing thereafter. Note that the maximum rate is obtained at a concentration of $MgCl_2$ 10-times lower than that of NaCl. This cannot be explained as arising from an ionic strength effect (which should only provide a factor of four), but rather suggests a specific role for Mg^{2+} cations (or, more properly, divalent cations in general [20]) in the interaction of Fld with the PSI membrane. This same behaviour has been reported for PSI-dependent Fd reduction [6], as well as for plastocyanin or cytochrome c_6 oxidation [16].

The possibility of studying *Anabaena* Fld photoreduction by *Anabaena* PSI particles was also investigated. PSI-enriched particles were prepared according to the method described for the cyanobacterium, *Synechocystis* [21]. However, we were not able to observe Fld reduction by laser flash spectroscopy in this system due to optical interferences, probably resulting from cyanobacterial pigments retained in the particles at the wavelengths used to follow Fld reduction. However, in steady-state experiments, which require lower PSI concentrations, electron transfer from *Anabaena* PSI to Fld can be observed (data not shown).

Several acidic residues in Fld have been reported to be involved in the interaction with FNR [22]. Since the gene of *Anabaena* sp. PCC 7119 Fld has been cloned and over-expressed in *E. coli* [23], and work on the preparation of Fld mutants is in progress, using the methods described here it will be possible to determine whether or not the same region of the Fld molecule interacts with both PSI and FNR. Furthermore, a comparative study of PSI reduction of Fd and Fld mutants will help to elucidate the factors which control these electron transfer processes. Such studies are presently underway.

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