Short Communication

Effect of Ferredoxin on the Diaphorase Activity of Cyanobacterial Ferredoxin-NADP Reductase¹

Received for publication June 20, 1984 and in revised form October 25, 1984

H. MELEND-HAREL, ELISHA TEL-OR*, AND ANTHONY SAN PIETRO
The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel (H.M.-H., E.T.-O.); and
Department of Biology, Indiana University, Bloomington, Indiana 47405 (A.S.P.)

ABSTRACT

The interaction of ferredoxin-NADP reductase (FNR) and ferredoxin (Fd) results in an enhanced rate of reaction and a shift of the pH optimum for the FNR-mediated diaphorase reaction. Low concentrations of NaCl (<100 millimolar), favorable for formation of the FNR:Fd complex, further magnify the alteration of the diaphorase reaction; the activity is enhanced 3-fold and pH optimum is shifted from 9.5 to 7.8. The Fd-stimulated diaphorase activity of FNR may result either from a conformational change of the enzyme and/or from a transition from a two electron to a one electron reaction.

A partially purified preparation of FNR was obtained by ion exchange chromatography on a diethylaminoethyl cellulose col-

The enzyme FNR² catalyzes the physiological photoreduction of NADP by PSI but exhibits also NADPH-Cyt c reductase and NADPH diaphorase activity; in the latter reaction an artificial oxidant, such as DCPIP or potassium ferricyanide, serves as the electron acceptor (2, 10, 19). The diaphorase reaction differs from the two other FNR-dependent reactions wherein the active catalyst is a 1:1 complex between FNR and Fd in a ratio of 1:1 (14, 15, 17). In previous studies, the effect of salt on the interaction between FNR and Fd resulted either in inhibition or stimulation of the diaphorase activity of FNR (5, 14). However, this effect was not further analyzed. In this report we describe two major changes in the diaphorase activity of FNR caused by its interaction with Fd; the activity is enhanced and the optimum pH of the reaction is lowered.

MATERIALS AND METHODS

Nostoc muscorum 7119 was obtained from D.I. Arnon, University of California, Berkeley. Cells were grown in Allen and Arnon medium (1) in 3-liter Erlenmeyer flasks at 27 ± 2°C with stirring and continuous illumination (cool white fluorescent light, I = 5 w·m⁻²). Cultures were harvested at late logarithmic phase of growth.

FNR Preparation and Assay. Washed cells were suspended in TMPK buffer, disrupted by sonication for about 1 min (Branson model W 140), and centrifuged at 30,000g for 30 min. The supernatant, adjusted to 1 mg/ml protein, was stored at −20°C and activity was retained for a few months.

¹ Supported by the United States-Israel Binational Research Fund.
² Abbreviations: FNR, ferredoxin-NADP reductase; DCPIP, dichlorophenolindophenol; TMPK buffer, 30 mM Tricine-NaOH (pH 7.5), 5 mM MgCl₂, 10 mM KCl, and 2 mM K₂HPO₄.

FIG. 1. The pH profile of the diaphorase reaction in presence of 40 mM NaCl with 11 μM Fd (●) and without Fd (○).

FIG. 2. The effect of Fd on the diaphorase reaction at pH 7.2 in presence of NaCl. The concentrations of NaCl were: 0 mM (○); 40 mM (Δ); 100 mM (▲); and 200 mM (●).
Table 1. Comparison of NADPH and NADH as Electron Donors for the FNR-Mediated Diaphorase Activity

<table>
<thead>
<tr>
<th></th>
<th>DCPIP Reduced</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 7.2</td>
<td></td>
</tr>
<tr>
<td>Fd, 11 μM</td>
<td>-Fd</td>
</tr>
<tr>
<td>NADPH, 33 μM</td>
<td>115</td>
</tr>
<tr>
<td>NADH, 33 μM</td>
<td>9.4</td>
</tr>
<tr>
<td>pH 9.0</td>
<td></td>
</tr>
<tr>
<td>Fd, 11 μM</td>
<td>-Fd</td>
</tr>
<tr>
<td>NADPH, 33 μM</td>
<td>60</td>
</tr>
<tr>
<td>NADH, 33 μM</td>
<td>4.2</td>
</tr>
</tbody>
</table>

of the reaction rate (Fig. 1). Further, the pH optimum for the reaction was shifted from pH 9.5 in the absence of Fd to pH 7.8 (in low salt medium; <40 mM NaCl). FNR-mediated diaphorase activity was measured at pH 7.2 as a function of Fd concentration in the presence of different concentrations of NaCl (Fig. 2). A gradual increase in FNR-mediated diaphorase activity was observed with 40 mM and 100 mM NaCl, whereas 200 mM NaCl inhibited the activity. A similar stimulation of FNR-mediated diaphorase activity by Fd at low salt concentration was reported by Nelson and Neumann (15), Bookmans et al. (3), and Nakamura and Kimura (13). In contrast, inhibition of FNR-mediated diaphorase activity in the presence of Fd was also reported (4, 14), and explained as competition between Fd and NADPH for the same binding site on the FNR.

The Fd-stimulated diaphorase activity of FNR is specific for NADPH; NADH is essentially inactive (Table I). When the FNR was purified further by ion exchange chromatography on diethylaminoethylcellulose, a similar pattern of the Fd-stimulated diaphorase activity of FNR was observed, and is shown in Figure 3.

A comparison of two activities catalyzed by FNR, NADPH Cyt c reduction, and NADPH diaphorase obtained with the partially purified FNR is presented in Figure 3. Both reactions responded similarly to NaCl and the highest activities were obtained at about 100 mM NaCl. Fd is involved catalytically in the Cyt c reduction and the pH optimum is 7.2 in the presence of low salt concentration (Melamed-Harel and Tel-Or, unpublished; see also 7, 12). The FNR-mediated diaphorase activity is higher at pH 9.0 in the absence of Fd (2, 9, 19) and at pH 7.2 in its presence. The results in Figure 3 demonstrate that both these effects of Fd are abolished at higher concentrations of salt (>0.3 M). It is known that the Fd:FNR complex is salt-sensitive and dissociates at high salt concentration (7, 15). These results clearly demonstrate, therefore, that interaction between FNR and Fd results in alteration of the properties of the diaphorase reaction catalyzed by FNR. The conditions optimal for the diaphorase reaction in the presence of Fd are similar to those required for the Cyt c reductase activity as well as the photoreduction of NADP during the final steps of the photosynthetic electron transport. Our possible explanation is that the interaction between Fd and FNR causes a change of the conformation of the FNR molecule and thereby affects its affinity for DCPIP. A similar explanation might also be valid for Cyt c reduction as well.

The mode of the electron transfer mediated by FNR in the diaphorase reaction is pH dependent; a two electron transfer reaction above 9.0 and a single electron transfer reaction below pH 9.0 (9). The mechanism of the diaphorase reaction is apparently modified by the FNR:Fd interaction such that the site for DCPIP reduction may be different at the higher pH optimum in the absence of Fd and the lower pH optimum in the presence of Fd. At low salt concentration, the pH optimum for the Cyt c reductase was shifted from pH 6.5 to 7.0 (Melamed-Harel and Tel-Or, unpublished) while the pH optimum for the Fd-stimulated diaphorase reaction was shifted from pH 8.5 to 7.8. These results suggest that the catalytic conformations of the FNR molecule in the Fd-stimulated diaphorase and Cyt c reductase are similar.

LITERATURE CITED

4. Davis DJ, A San Pietro 1977 Interaction between spinach ferredoxin and

RESULTS AND DISCUSSION

The addition of a saturating concentration of Fd (11 μM) to the FNR-mediated diaphorase reaction resulted in enhancement


