Structural aspects of plant ferredoxin : NADP$^+$ oxidoreductases

P. Andrew Karplus* & H. Richard Faber

Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331, USA; *Author for correspondence (e-mail: karplusp@science.oregonstate.edu; fax: +1-541-737-0481)

Received 30 April 2004; accepted 24 May 2004

Key words: evolution, ferredoxin NADP reductase, photosynthesis, protein crystallography, protein structure

Abstract

Ferredoxin reductase (FNR) is ubiquitous among photosynthetic organisms as the enzyme directly responsible for the generation of NADPH. Structural studies over the last 15 years have generated over 30 crystal structures of wild-type and mutant FNRs that have yielded a great deal of insight into its structure–function relations. These insights are summarized and combined to propose a structurally informed cycle for FNR catalysis in vivo.

Abbreviations: Fd – ferredoxin; FNR – ferredoxin : NADP reductase; PAMP – 2$'$/5$'$-AMP

Scope and purpose

Three-dimensional structural information not only plays a large role in supporting our understanding of protein function at the molecular level, but when it comes to how proteins do their jobs, it is not an exaggeration to say that structure is function: given a structure, the function flows from it. The richest understanding of structure–function relations, however, derives from a combination of extensive experimental studies of function and experimental determinations of each of the structural states relevant to function. Fortunately, for plastid type ferredoxin : NADP$^+$ oxidoreductases (ferredoxin reductase; FNR), the enzyme responsible for the generation of NADPH during photosynthesis, these criteria are rather well fulfilled. Since its discovery four independent times (Avron and Jagendorf 1956; Keister et al. 1960; Shin et al. 1963; Zanetti and Forti 1966), FNR has been the subject of many biochemical studies, and, beginning with the structure determination of spinach FNR (Karplus et al. 1984, 1991), crystallographic studies have provided a wealth of structural information. Since this review focuses on a synthesis of the currently available structural information, it is not comprehensive in its citation of the literature, and readers are referred to a number of excellent earlier reviews for a greater discussion of specific results and an entry into much of the literature (Karplus and Bruns 1994; Gomez-Moreno et al. 1996; Arakaki et al. 1997; Hurley et al. 2002; Carrillo and Ceccarelli 2003).

In this review, we will summarize the structural information that exists for FNRs and the insights into structure–function relations that have been generated through the study of the wild type enzymes and their site directed mutants. In addition, because the study of related proteins can often generate additional insights, we will briefly summarize the structural information available for other enzymes that incorporate a module related to FNR. The details of the FNR : ferredoxin interaction are not presented here, because those are the focus of a separate article in this issue (Hanke et al. 2004).
Introduction

Plastid type FNRs include those ferredoxin reductases that are responsible for the production of NADPH during photosynthesis, both those located in the chloroplasts of higher plants or algae, and those in cyanobacteria. Because of their close similarity, also included are higher plant FNRs that are present in the plastids of non-photosynthetic tissues such as roots. Both enzymes catalyze the reaction

$$2\text{Fd}_{\text{red}} + \text{NADP}^+ + \text{H}^+ \leftrightarrow 2\text{Fd}_{\text{ox}} + \text{NADPH}$$

(Scheme 1)

where \(\text{Fd}_{\text{red}}\) is reduced ferredoxin and \(\text{Fd}_{\text{ox}}\) is oxidized ferredoxin, with the physiologically relevant direction of enzymes involved in photosynthesis being to the right (producing NADPH) and the physiologically relevant direction of the other enzymes, such as those in root plastids, being to the left (using NADPH to produce reduced ferredoxin for use in nitrate reduction and other purposes). The redox potentials of the FNRs working in opposite directions appear to be tuned for their function (Aliverti et al. 2001).

All of the plastid type FNRs share sequence identities of over 40%, with the next most similar protein being a non-photosynthetic FNR from the apicoplast of \(\text{T. gondii}\) at about 35% identity (Vollmer et al. 2001). Since the apicoplast is thought to have evolved from the chloroplast of an engulfed alga, this enzyme (and other even more distantly related enzymes) could be included as plastid type FNRs, but based on its significantly lower sequence similarity, we have chosen to exclude it by our definition. The plastid FNRs considered in this review can be seen to group in three major branches (Figure 1). The higher plant leaf and more distantly related glaucocyst FNRs are on one branch, the widely diverse cyanobacterial FNRs are a second branch, and the root plastid enzymes together with the enzymes from green algal chloroplasts form the third branch. The closer similarity of the root enzymes to the enzymes from green algal chloroplasts (65% identity) has been noted before (Choi et al. 1996). Consistent with the relatively recent appearance of higher plants in an evolutionary time frame, the leaf and root enzymes each form rather tight clusters (<20% sequence variation). Further discussion of these evolutionary relationships and also those with the more distantly related bacterial FNRs can be found in Ceccarelli et al. (2004).

![Figure 1](image_url)