Biochimica et Biophysica Acta xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Dynamics of the active site architecture in plant-type ferredoxin-NADP$^+$ reductase catalytic complexes

Ana Sánchez-Azqueta [a,b], Daniela L. Catalano-Dupuy [c], Arleth López-Rivero [c], María Laura Tondo [c], Elena G. Orellano [c], Eduardo A. Ceccarelli [c], Milagros Medina [a,*]

[a] Departamento de Bioquímica y Biología Molecular y Celular, Facultad de Ciencias, Universidad de Zaragoza, Zaragoza, Spain
[b] Instituto de Biocomputación y Física de Sistemas Complejos (BIFI), Unidad Asociada BIFI-IQFR (CSIC), Universidad de Zaragoza, Zaragoza, Spain
[c] Instituto de Biología Molecular y Celular de Rosario, CONICET, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Santa Fe, Argentina

ARTICLE INFO

Article history:
Received 24 March 2014
Received in revised form 5 June 2014
Accepted 11 June 2014
Available online xxxx

Keywords:
Ferredoxin-NADP$^+$ reductase
Flavoenzyme
Kinetic isotope effect
Hydride transfer
Charge–transfer complex
Plastidic-type FNR
Bacterial-type FPR

ABSTRACT

Kinetic isotope effects in reactions involving hydride transfer and their temperature dependence are powerful tools to explore dynamics of enzyme catalytic sites. In plant-type ferredoxin-NADP$^+$ reductases the FAD cofactor exchanges a hydride with the NADP(H) coenzyme. Rates for these processes are considerably faster for the plastidic members (FNR) of the family than for those belonging to the bacterial class (FPR). Hydride transfer (HT) and deuteride transfer (DT) rates for the NADP$^+$ coenzyme reduction of four plant-type FNRs (two representatives of the plastidic type FNRs and the other two from the bacterial class), and their temperature dependences are here examined applying a full tunnelling model with coupled environmental fluctuations. Parameters for the two plastidic FNRs confirm a tunnelling reaction with active dynamics contributions, but isotope effects on Arrhenius factors indicate a larger contribution for donor–acceptor distance (DAD) dynamics in the *Pisum sativum* FNR reaction than in the *Anabaena* FNR reaction. On the other hand, parameters for bacterial FPRs are consistent with passive environmental reorganisation movements dominating the HT coordinate and no contribution of DAD sampling or gating fluctuations. This indicates that active sites of FPRs are more organised and rigid than those of FNRs. These differences must be due to adaptation of the active sites and catalytic mechanisms to fulfil their particular metabolic roles, establishing a compromise between protein flexibility and functional optimisation. Analysis of site-directed mutants in plastidic enzymes additionally indicates the requirement of a minimal optimal architecture in the catalytic complex to provide a favourable gating contribution.

1. Introduction

Plant-type ferredoxin-NADP$^+$ reductases (FNRs) have evolved from a common ancestor into two classes: plastidic FNRs, which are found in plastids of plants, algae and cyanobacteria, and bacterial FNRs, herein known as FPRs [1,2]. The main role of plastidic FNR is the photosynthetic transfer of reduction equivalents from ferredoxin (Fd) to NADP$^+$ via its non-covalently bound FAD redox cofactor, although in vivo this reaction is reversible and FNR can provide electrons to different electron carrier proteins using NADPH as reductant [3]. The bacterial counterparts catalyse the non-photosynthetic reaction providing reducing power to detoxification and nitrogen fixation processes [1,2,4,5]. Differences in the requirements of their biological functions are reflected in turnover rates, substrate affinity and specificity, and, therefore, catalytic efficiency, as a result of the divergence achieved along functional specialisation. The lower catalytic efficiency of FPRs is related to structural differences in the isoalloxazine active site environment: i) the FAD acquires an extended conformation in plastidic FNRs but it is folded in FPRs and, ii) the isoalloxazine stacks between two Tyr residues (Tyr79 and the C-terminal Tyr303 in *Anabaena* (*An*FNR)) in plastidic FNRs, while FPRs

Abbreviations: FNR, ferredoxin-NADP$^+$ reductase; FPR, bacterial-type FNR; *An*FNR, FNR from the cyanobacterium *Anabaena* PCC 7119; *Ps*FNR, FNR from *Pisum sativum*; *Xa*FPR, FPR from *Xanthomonas axonopodis* pv. *citri*; *Ec*FPR, FPR from *Escherichia coli*; FNR_{ox}, FNR in the fully oxidised state; FNR_{hq}, FNR in the anionic hydroquinone (fully reduced) state; HT, hydride transfer; DT, deuteride transfer; WT, wild-type; CTC, charge–transfer complex; CTC-1, FNR_{ox}:NADPH CTC; CTC-2, FNR_{hq}:NADP$^+$ CTC; 2′-P-AMP, 2′-P-AMP moiety of NADP(H); N5-FAD, N5 hydride donor/acceptor of the FADH$^-$/FAD isoalloxazine ring of FNR; C4-NADP(H), C4 hydride acceptor/donor of the NADP$^+$/NADPH nicotinamide ring; NADPD, (4R)-4-^{2}H-NADPH; $k_{A\rightarrow B}$, $k_{B\rightarrow C}$, apparent/observed rate constants obtained by global analysis of spectral kinetic data; k_{HT}, k_{DT}, k_{obsHT}, k_{obsDT}, limiting hydride and deuteride transfer first-order rate constants for the reduction of FNR and their corresponding observed values under particular conditions; KIE, kinetic isotope effect on rate constants; A_H, A_D, Arrhenius pre-exponential factors for hydride and deuteride, respectively; E_{aH}, E_{aD}, activation energies for hydride transfer and deuteride transfer, respectively; DAD, donor–acceptor distance

* Corresponding author at: Departamento de Bioquímica y Biología Molecular y Celular, Facultad de Ciencias, Universidad de Zaragoza, E-50009 Zaragoza, Spain. Tel.: +34 976 762476; fax: +34 976 762123.
E-mail address: mmedina@unizar.es (M. Medina).

http://dx.doi.org/10.1016/j.bbabio.2014.06.003
0005-2728/

have a C-terminal extension where the residue facing the isoalloxazine ring moiety is not the C-terminal [4,6–8] (Fig. 1). The length and sequence of this extension are not homogeneous among FPRs. Subclass I, including *Rhodobacter capsulatus* (*Rc*FPR) and *Xanthomonas axonopodis* pv. *citri* (*Xa*FPR) enzymes among others, has an Ala instead of the plastidic C-terminal Tyr followed by a Phe and up to 5 additional residues [9–12]. Members of subclass II maintain a Tyr stacking the *re*-face of the isoalloxazine and it is followed by a Trp, as in *Escherichia coli* FPR (*Ec*FPR) [13–15]. In both subclasses, the abovementioned Phe or Trp residue located at the C-terminal extension stacks on the adenosine moiety of FAD, apparently contributing to its folded conformation.

In plastidic FNRs the hydride transfer (HT) takes place between the N5 atom of the FAD(H^-) isoalloxazine (N5-FAD) and the C4 atom of the nicotinamide ring of NADP(H) (C4-NADP(H)) [16,17]. Mutational and theoretical analysis focused on the displacement of the Tyr stacking on the *re*-face of the isoalloxazine, required to allow the approaching of the reacting atoms during HT, and have contributed to understand mechanistic details of this process as well as the role of the Tyr itself [17–26]. Though the presence of this Tyr is not obligatory for HT, it is crucial for the high catalytic efficiency of FNRs. It modulates the FAD midpoint reduction potential, avoids a too strong interaction between the reacting rings that would be incompatible with product release, and contributes to the optimal geometry between the reacting atoms for HT, N5-FAD and C4-NADP(H). Finally, it provides the active site with the required flexibility to allow the HT step occurring through tunnelling [21,22]. It is accepted that in bacterial FPRs the HT also takes place between C4-NADP(H) and N5-FAD. However, differences in the side-chain stacking against the isoalloxazine and, particularly, the presence of the C-terminal extension suggest a more complex mechanism to attain the catalytically competent interaction (Fig. 1) [6,7,11,15,27]. So far, no details about structural arrangement and dynamics of the active site during catalysis in FPRs have been provided.

The importance of slow (ms to s) protein flexibility in substrate recognition and allosterism is widely accepted [28]. Faster (fs to ps) motions coupled to the chemical step have also been pointed as contributing to active site dynamics in enzyme catalysed reactions. Variations of the Eyring's Transition State Theory have been used to treat enzyme-catalysed reactions, including fast electron–proton coupled transfers [29–39]. The most recent approaches postulate HT processes as fully quantum-mechanical events modulated by dynamical motions of the active site environment within the "environmentally coupled full tunnelling model" that describes two types of protein motions putatively linked to catalysis: pre-organisation and reorganisation [40]. Pre-organisation motions are assumed to occur prior to the HT event, in the ps to ns time scale, and involve large regions of the protein. Reorganisation motions involve heavy atoms within the active site, and constitute fast (ps to fs) nuclear fluctuations. Despite evidences for a role of protein dynamics in accelerating HT reactions [41–44], their contribution to catalysis is still on debate [45]. Studies on dihydrofolate reductases and on some pyridine-nucleotide dependent

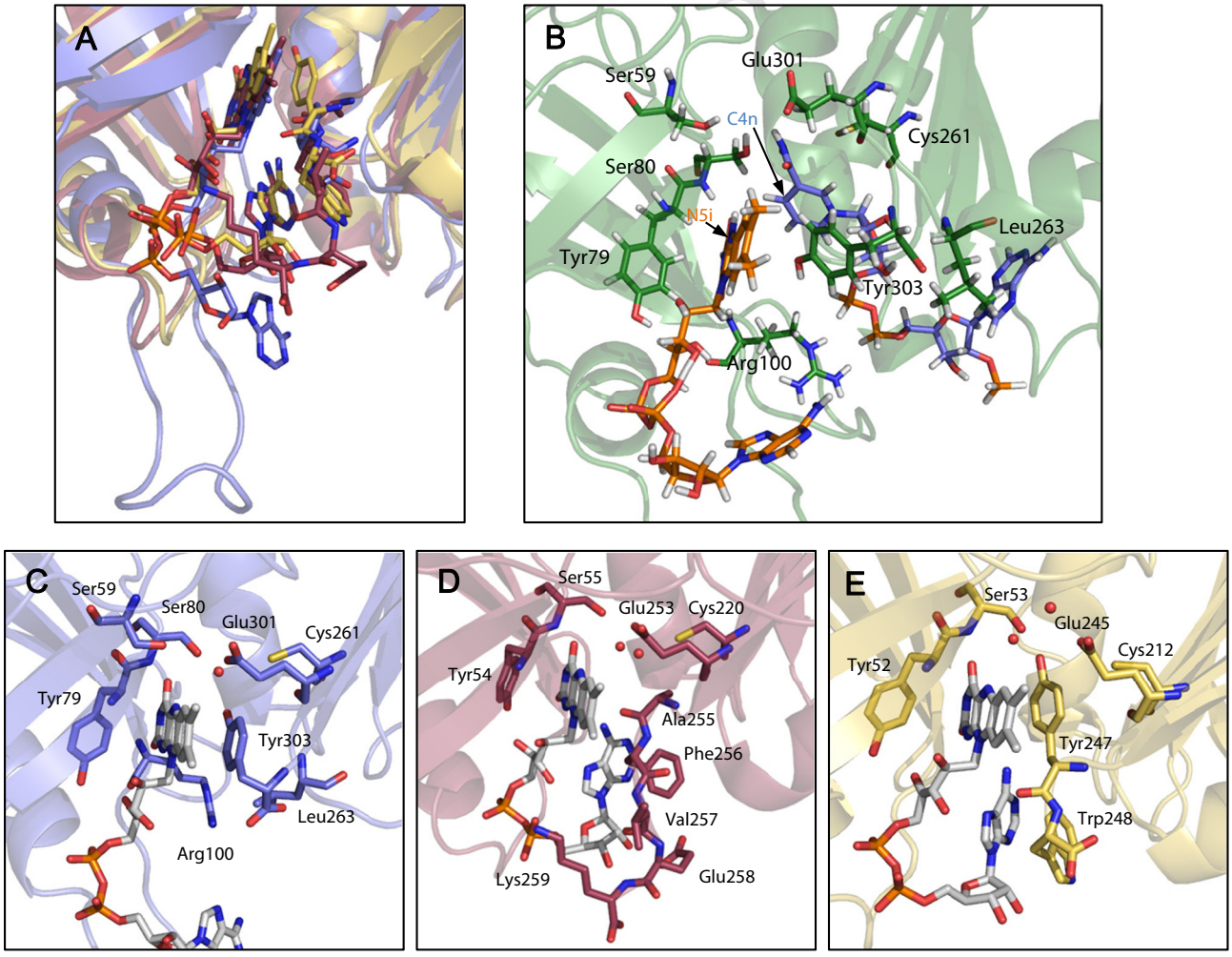

Fig. 1. The FAD environment in the plant-type FNR family. (A) Comparison of the FAD folding and environment in the crystal structures of *An*FNR (PDB 1que, blue), *Xa*FPR (PDB 4b4d, wine red) and *Ec*FPR (PDB 1fdr, yellow). (B) Theoretical model of the allocation of the nicotinamide moiety of $NADP^+$ in the active site of $AnFNR_{hq}$ as obtained by MD simulations [23]. Protein chain is shown in green, FAD in orange and NADPH in blue. Detail of the active site configuration in (C) *An*FNR, (D) *Xa*FPR and (E) *Ec*FPR. Key residues are shown as CPK coloured sticks.

Please cite this article as: A. Sánchez-Azqueta, et al., Dynamics of the active site architecture in plant-type ferredoxin-$NADP^+$ reductase catalytic complexes, Biochim. Biophys. Acta (2014), http://dx.doi.org/10.1016/j.bbabio.2014.06.003