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Why WrbA is weaker than flavodoxin in binding FMN. A molecular modeling study

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Abstract

The protein WrbA from *Escherichia coli* is the founding member of a class of novel multimeric flavodoxin-like proteins implicated in defense against oxidative stress. Although, WrbA is predicted to share the twisted α/β open-sheet fold of the flavodoxins and to bind flavin mononucleotide (FMN) as its physiological cofactor, the binding is much weaker in comparison with flavodoxin (the binding constants are ~2 μM for WrbA and ~1 nM for flavodoxin). To elucidate the different FMN-binding behaviors of WrbA and flavodoxin, we modeled the WrbA structure and examined its interactions with FMN by docking experiments, and then compared them with those at the flavodoxin active site. The results provide a rationale for the reduced cofactor affinity displayed by WrbA relative to flavodoxin.

Keywords: Homology modeling; Docking calculations; Protein evolution; Hydrogen bonds; Hydrophobic interactions

1. Introduction

The protein WrbA from *Escherichia coli* (*E. coli*) is the founding member of a class of novel multimeric flavodoxin-like proteins implicated in defense against oxidative stress [1,2]. Whereas WrbA is predicted to share the twisted α/β open-sheet fold of the flavodoxins and to bind flavin mononucleotide (FMN) as its physiological cofactor [2,3], the binding is much weaker (the binding constants for WrbA and flavodoxin are ~2 μM and ~1 nM, respectively) although still specific [3,4].

The remote homology to flavodoxins previously led to a prediction of the overall architecture of WrbA [2]. However, the different FMN-binding behaviors of WrbA and flavodoxin remain elusive. Recently, the crystal structure of the WrbA protein from *Deinococcus radiodurans* was determined (PDB code: 1YRH) [5]. This protein displays 53% sequence identity to the *E. coli* WrbA, and therefore provides a good template for high-accuracy modeling. In this work, we undertook such modeling and then employed the derived structure for docking experiments with FMN. The results are discussed with reference to the differences in affinity between WrbA and flavodoxin.

2. Methods

2.1. Structure

Structure coordinates for *D. radiodurans* WrbA in PDB file 1YRH:A with the ligand FMN deleted were used to develop the *E. coli* WrbA model employing the homology modeling module of Insight II software [6]. Molecular dynamics (MD) equilibration was performed with the consistent-valence force field (CVFF) [7] on an SGI Origin 350 server with 8 CPUs. The model was minimized by 500 conjugate gradient steps for equilibration, heated from 2 to 300 K during 35 ps at a temperature increment of 50 K per 5 ps, then the constant temperature and pressure algorithm was applied at 300 K for

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50 ps. The velocity verlet integrator was used with an integration step of 2 fs.

2.2. Ligand docking and energy analysis

Standard parameters of the program FlexX [8], as implemented in the molecular modeling software SYBYL 7.0 [9], were used to explore the binding modes of FMN. The Ludi module of Insight II was used to evaluate binding affinities. This method can estimate the non-bonding interactions between receptor and ligand. The Ludi score derived by the program is empirically related to the dissociation constant K_d: Ludi score $= -100 \log K_d$. Non-bonding interaction energies (sum of coulombic and van der Waals interaction energies) were calculated using the Discover module of Insight II.

3. Results

3.1. Structural model for WrbA

The apoprotein structure for *E. coli* WrbA was modeled by homology using the crystal structure of *D. radiodurans* WrbA (1YRH:A) as template, with ligand FMN deleted. The model was then equilibrated using the discover module with a consistent-valence force field. The resulting predicted structure for *E. coli* WrbA is shown in Fig. 1. The fundamental structural features of the flavodoxin fold can be recognized, namely, a central five-stranded parallel hydrophobic β-sheet sandwiched between two pairs of amphipathic α-helices. The modeled structure is consistent with that predicted by sequence similarity between WrbA and flavodoxin [2]. Evaluation of the model performed with the profile-3D module of Insight II yields positive compatibility scores for all residues (Fig. 2a), indicating that the sequence of *E. coli* WrbA is energetically compatible with the structural environment of the model throughout the structure.

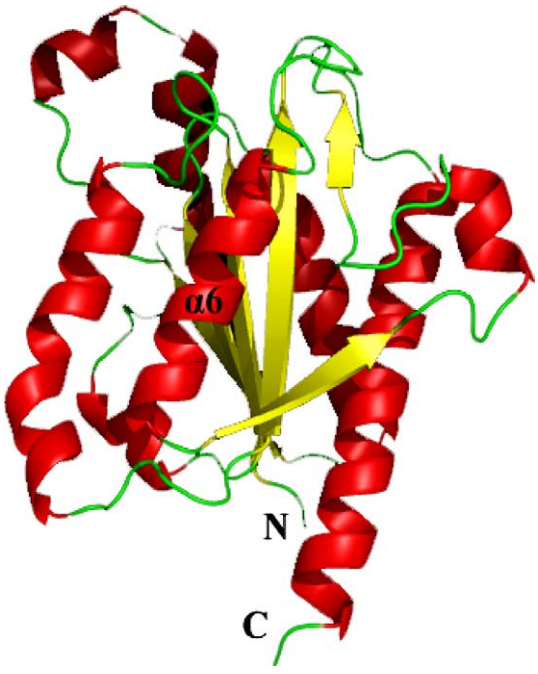

Fig. 1. Cartoon of the model structure of the *E. coli* WrbA apoprotein obtained using *D. radiodurans* WrbA apoprotein as a template. N- and C-termini and α1 are labeled.

The sequence alignment to *E. coli* flavodoxin and *D. radiodurans* WrbA derived from structure superimposition is shown in Fig. 2b. Previous studies [2] by sequence similarity identified a region of high sequence similarity within the WrbA family (Gly116-Pro139) and low sequence similarity to flavodoxin that was suggested to be responsible for oligomerization, a peculiar feature of the WrbA family. As predicted by that analysis, this element folds in an alpha-turn-beta conformation and the helix mediates subunit contacts in the tetrameric unit of 1YRH. However, in contrast to the previous alignment, this helix turns out to correspond to α3 of flavodoxin. The *E. coli* flavodoxin belong to the group of the long-chain flavodoxins, whose last beta strand (β5) is interrupted by a short helix. The proteins WrbA appear to share this structural feature.

3.2. Ligand docking to flavodoxin

The docking program FlexX was used to predict the binding modes of oxidized FMN to flavodoxin and WrbA. This software uses a fast incremental construction algorithm to place ligands into an active site. To first evaluate the predictive power of this method using a known structure, binding modes were examined for *E. coli* flavodoxin.

Starting with the coordinates of only the protein moiety in PDB file 1AHN [10], the results of docking oxidized FMN indicate that the predicted orientation, conformation, and protein contacts are very similar to those found in the holoflavodoxin crystal structure (Fig. 3 and Table 1). The modeled flavodoxin–FMN complex is shown in Fig. 3a. The root-mean-square deviation (RMSD) between heavy atoms of the FMN ligand in the docking-derived and the X-ray structure is 1.21 Å, after optimal superimposition of protein atoms (Fig. 3b). The binding affinity calculated for the FMN/flavodoxin model from the Ludi score (827, see Section 2) is 5.4 nM, in the same order of magnitude as the experimentally determined value of ~1 nM [4]. Assignment of the non-bonding interaction energy by FlexX to coulombic and van der Waals components is given in Table 2.

In the flavodoxin/FMN complex predicted by docking, 16 hydrogen bonds form between protein residues of the flavodoxin and the FMN. Of these, Thr12, Thr56 and Asp147 are with the ribityl tail, Thr59, Gly60, Asp90, Tyr97 and Cys99 with the isoalloxazine ring, Ser10, Asp11, Thr12, Asn14 and Trp57 with the phosphate group. Furthermore, the isoalloxazine ring is sandwiched between the side chains of Trp57 and Tyr94 and surrounded by several other hydrophobic residues (Fig. 3c). The crystal structure of the flavodoxin/FMN complex from PDB file 1AHN is shown in Fig. 3d for comparison. The hydrogen bonds formed between the protein and FMN (crystal and docking-derived structures) are listed in Table 1. The protein–ligand interactions are very similar in the two cases, supporting the accuracy of the present docking method.