Structure and Site-directed Mutagenesis of a Flavoprotein from <i>Escherichia coli</i> That Reduces Nitrocompounds

ALTERNATION OF PYRIDE NUCLEOTIDE BINDING BY A SINGLE AMINO ACID SUBSTITUTION*

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The crystal structure of a major oxygen-insensitive nitroreductase (NfsA) from <i>Escherichia coli</i> has been solved by the molecular replacement method at 1.7-Å resolution. This enzyme is a homodimeric flavoprotein with one FMN cofactor per monomer and catalyzes reduction of nitrocompounds using NADPH. The structure exhibits an α + β-fold, and is comprised of a central domain and an excursion domain. The overall structure of NfsA is similar to the NADPH-dependent flavin reductase of <i>Vibrio harveyi</i>, despite definite difference in the spatial arrangement of residues around the putative substrate-binding site. On the basis of the crystal structure of NfsA and its alignment with the V. harveyi flavin reductase and the NADPH-dependent nitro/flavin reductase of <i>Bacillus subtilis</i>, residues Arg203 and Arg208 of the loop region between helices I and J in the vicinity of the catalytic center FMN is predicted as a determinant for NADPH binding. The R203A mutant results in a 33-fold increase in the <i>K<sub>m</sub></i> value for NADPH indicating that the side chain of Arg203 plays a key role in binding NADPH possibly to interact with the 2'-phosphate group.

Nitroaromatic compounds including nitrofurans, nitropyrenes, and nitrobenzenes have been used as antimicrobial agents, food additives and raw materials in several industrial processes (1–5), and as a result are distributed widely around the environment. Many of these compounds are toxic, mutagenic, or carcinogenic (6–8). It is believed that enzymatic transformation is needed for nitroaromatic compounds to show these serious effects (9, 10). The reduction of a nitro group of a parent nitrocompound is a key step of this process (11, 12). Enzymes, which catalyze the reduction of nitrocompounds using a reduced pyridine nucleotide, are termed nitroreductases and are distinguished by their sensitivity of activity to oxygen (9, 10).

The oxygen-sensitive enzymes can catalyze nitroreduction only under anaerobic conditions. A nitro-anion radical formed by a one-electron transfer is immediately reoxidized in the presence of oxygen to a parent nitrocompound and superoxide (13, 14). In this futile cycle, reducing equivalents are consumed without the progress of nitroreduction and nitrocompounds perform as a catalyst to reduce oxygen. On the other hand, the oxygen-insensitive enzymes catalyze an obligatory two-electron reduction. A nitro group of a parent nitrocompound is reduced by a series of two-electron transfers, through nitroso and hydroxylamine intermediates, and finally to an amino group (13). The hydroxylamine intermediate arising from the four-electron transfer in total is found to be toxic, carcinogenic, or mutagenic.

Three proteins with oxygen-insensitive nitroreductase activity in <i>Escherichia coli</i> have been identified (15). NfsA is the major component, while NfsB and NfsC are minor components. NfsA and NfsB have been well studied relative to NfsC. NfsA and NfsB have similar enzymatic property, although NfsA has only 7% identity with NfsB on the amino acid sequence alignment. Both NfsA and NfsB are flavoenzymes with FMN as the prosthetic group and catalyze the reduction of nitrocompounds by Ping Pong Bi Bi kinetics (16, 17). Counterparts of NfsA and NfsB, found in luminescent bacteria (16, 17), are flavin reductase (FRP) of <i>Vibrio harveyi</i> (18) and flavin reductase (FRase I) of <i>Vibrio Fischeri</i> (19), respectively. Enzyme that resembles FRP in the amino acid sequence alignment is also found in <i>Bacillus subtilis</i> and is called NfrA1 (20). A comparison of

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The abbreviations used are: NfsA, the major oxygen-insensitive NADPH-dependent nitroreductase of <i>Escherichia coli</i>; NfsB, the minor oxygen-insensitive NAD(P)H-dependent nitroreductase of <i>E. coli</i>; FRP, an NADPH-dependent flavin reductase of <i>V. harveyi</i>; NOX, an NADH oxidase of <i>T. thermophilus</i>; NfrA1, an NADPH-dependent nitro/flavin reductase of <i>B. subtilis</i>; FRase I, the major NAD(P)H:FMN:oxidoreductase of <i>V. Fischeri</i>; r.m.s., root mean square.
Rigaku imaging plate detector system, R-AXIS IIc, with double mirror-A data set to 2.3-Å resolution was recorded from single crystals using a were collected by using both laboratory and synchrotron x-ray sources. To obtain a high-resolution data set with high completeness, data sets of one dimer per unit cell, the calculated V isopropyl alcohol as an additive in 100 mM MES buffer (pH 6.5). Crys-

**EXPERIMENTAL PROCEDURES**

**Protein Purification and Crystallization**—Recombinant E. coli NfsA was expressed and purified as described previously (28). Crystals of NfsA were obtained using the hanging drop vapor diffusion method, was expressed and purified as described previously (26). Crystals of 

### TABLE I

| Data collection | Resolution (Å) | 1.7 |
|-----------------|----------------|-----|
| No. of reflections | 41935         |     |
| Completeness (%) | 91.8          |     |
| Rmerge<sup>a</sup> | 0.033         |     |
| Refinement statistics |               |     |
| R<sub>cryst</sub><sup>b</sup> (%) | 18.9         |     |
| R<sub>free</sub> (%) | 20.6         |     |
| No. of solvent molecule | 349          |     |
| Average B factor (Å²) |             |     |
| Main chain | 7.44         |     |
| Side chain | 10.22        |     |
| Overall | 9.34         |     |
| Stereochemistry |               |     |
| r.m.s.d bond length (Å) | 0.009        |     |
| r.m.s.d bond angle (°) | 1.745        |     |
| r.m.s.d dihedral (°) | 21.918       |     |
| r.m.s.d improper (°) | 0.759        |     |

<sup>a</sup> R<sub>merge</sub> = Σ|I<sub>hkl</sub>−<I><sub>hkl</sub>|/Σ<I><sub>hkl</sub>, where <I><sub>hkl</sub> is the average of individual measurements of I<sub>hkl</sub>.<sup>b</sup> R<sub>cryst</sub> = Σ|Fo−Fc|/ΣFo, where |Fo|<sub>hkl</sub> is calculated using about 5% of reflections omitted from the refinement.

### TABLE II

| Electron donor | NADPH | NADH |
|----------------|-------|------|
| Wild-type | 4.5 | 0.07 |
| R203A | 0.3  | 0.06 |
| R208A | 4.1  | 0.06 |

Organization (Tsukuba, Japan). Data frames were collected using the Weissenberg camera for macromolecular crystallography, an imaging plate, and a Fuji image reader (BAS2000) and were processed using the data reduction programs DENZO and SCALEPACK (27). To collect the diffraction data efficiently, both Weissenberg and oscillation photographs were used, due to the difficulty to align crystals for the Weissenberg method under cryo-cooling conditions.

**Structure Determination and Refinement**—The FRP molecule (21) was used as the search model for molecular replacement. The rotation function was calculated with AMORE (28) and gave a single solution with a correlation coefficient of 36.9%. To improve the accuracy of the solution, the resultant structure was subjected to 5 cycles of rigid body refinement using data between 4.0- and 10.0-Å resolution. The crystallographic R-factor and correlation coefficient was refined to 43.6 and 38.8%, respectively. To improve F<sub>o</sub> and phases, the following procedure was performed several times. The resulting F<sub>e</sub>, F<sub>c</sub>, and phases were subjected to density modification procedures with solvent flattening, histogram matching, and averaging using DM (29). The improved F<sub>e</sub> and phases were used to calculate a SIGMAA weighted F<sub>e</sub> − F<sub>c</sub> electron density map (30). The density map was displayed in the program O (31) and used to rebuild the model manually.

Further improvement of the model was accomplished by using alternate cycles of refinement in the program X-PLOR (32, 33) and CNS (35), and manual intervention using O. Initially strict noncrystallographic symmetry was imposed on the model followed by noncrystallographic symmetry restraints. Approximately 5% of reflections from a number of thin shells were selected to calculate the free R-factor, using SPODOLS. The first round of refinement consisted of positional refinement and simulated annealing beginning at 3000 K. Later steps of refinement included the refinement of grouped or individual temperature factors. Simulated annealing omit maps were used to check and rebuild the region including residues 202 to 209 in the model. Water molecules were identified by searching difference maps using WATPEAK in the CCP4 package (34) and proline are in the most favored regions of the Ramachandran plot and the rest of the residues except for Asn<sup>179</sup> are in the additional allowed regions. Asn<sup>179</sup> is located in a tight turn between b-strand and a-helix. The overall geometry (36) is good as compared with proteins of the same resolution. R.m.s deviations of distances and angles are 0.009 Å and 1.745°, respectively. Crystallographic data and refinement statistics are shown in Table I.

### Construction and Expression of Mutants—Mutants were prepared using the polymerase chain reaction (37) with the Quick Change Site-directed Mutagenesis Kit (Stratagene). A plasmid (pAJ102) containing the nfsa gene was constructed previously (38). Synthetic oligonucleotides containing the desired mutation were described as used in mutagenic primers, 5'-TAATTCACAGGTGTCGATG-3' and 5'-AGGATTTTCCTGCGGCTG-3'.

Mutagenesis was confirmed by dyeoxy sequencing of mutated genes using a Shimadzu DNA sequencer, DSQ-1000. The mutant NfsA were expressed and purified, yielding a single band on SDS-polyacrylamide gel electrophoresis according to the methods reported previously (26).

### Kinetic Characterization of the Mutants—Cytosolic fractions were prepared from overnight cultures of E. coli clones containing wild-type and mutant plasmid constructs. To measure the relative activity of wild-type enzyme and two mutants, 100 μM of either NADPH or NADH was used as an electron donor and 100 μM nitrofurazone was used as an electron acceptor (Table II). Steady-state kinetic analyses were carried out by monitoring consumption of NADPH by the decrease in absorbance at 400 nm (ε = 12.96 μM cm<sup>−1</sup>) (13) in reaction mixtures containing appropriate amounts of the purified enzymes in 50 mM Tris-HCl buffer, pH 7.0, at 23 °C. To determine the K<sub>m</sub> values for both NADPH and nitrofurazone, the initial rates of the reactions were observed at varying concentrations of NADPH in the range of 5 to 200 μM

<sup>2</sup> B. Hazes, unpublished results.
for each of the various nitrofurazone concentrations in the range of 5–200 μM. NADPH in the reaction mixture was not saturating to determine the $K_m$ of R203A mutant for NADPH. It was difficult to obtain accurate kinetic data when using more than 200 μM NADPH, since the absorption curves of both substrates overlapped each other near the measured wavelength. An estimate of the $K_m$ for NADPH and the value for $V_{max}$ was obtained by extrapolation for this mutant. The analysis of the data was performed using the program Cleland (39).

RESULTS

Description of the NfsA Structure—NfsA is a homodimeric globular protein of about 45 Å in diameter that contains one FMN per monomer as a cofactor (Fig. 1). An NfsA molecule consists of an $\alpha + \beta$-fold formed by 12 $\alpha$-helices and five $\beta$-strands and is divided into two domains, the central domain and the excursion domain. The central domain is composed of residues 15–161 and 226–240, and forms an anti-parallel $\beta$-sheet structure comprised of strands 1–4. On one side of the central $\beta$-sheet, helices C, D, and G lie almost vertically relative to the $\beta$-sheet, and on the other side, the sheet is surrounded by helices B and F (Fig. 1). The excursion domain consists of residues 1–14 of one monomer and residues 162–225 of the other monomer. Residues 1–14 form helix A, which contacts with helices B and F in the central domain. Residues 162–225 forms a long coil element (residues 162–171) followed by helix H and strand 5, which is parallel to strand 1 in the central domain (Fig. 1) and connect helix I through a tight turn. A loop (residues 202–211) and helix J consists of residues 15–161 and 226–240, and forms an anti-parallel $\beta$-sheet, helices C, D, and G lie almost vertically relative to the $\beta$-sheet, and on the other side, the sheet is surrounded by helices B and F (Fig. 1). The excursion domain consist of residues 1–14 of one monomer and residues 162–225 of the other monomer. Residues 162–225 forms a long coil element (residues 162–171) followed by helix H and strand 5, which is parallel to strand 1 in the central domain (Fig. 1) and connect helix I through a tight turn. A loop (residues 202–211) and helix J.

As shown in Fig. 2A, the $2F_o - F_e$ electron density map is of high quality at the interface of both monomers in the NfsA crystal structure. The re face of the isoalloxazine portion of cofactor FMN faces the putative substrate-binding site. The average B-factor for two FMNs is 4.3 Å$^2$, consistent with FMN being bound tightly through many hydrogen bonds provided by surrounding main chain and side chain atoms of the peptide chain (Fig. 2B).

The dimethylbenzene portion of the isoalloxazine group is stabilized by hydrophobic interaction with helix F in both monomers (Fig. 1). On the other hand, the O2A/O4A side is partly exposed to the solvent. The O2A atom forms a hydrogen bond to the N-ε of Arg$^{15}$ and a water molecule. The O4A atom forms hydrogen bonds to the amide nitrogens of Gly$^{130}$ and Gly$^{131}$, and the Nε-2 atom of Gln$^{67}$. The N-1 and N-5 atoms, respectively. The redox site of FMN, have hydrogen bonds with the Nγ-2 atom of Arg$^{15}$ and the amide nitro of Gly$^{130}$, respectively. On the si face of FMN, the carbonyl oxygen of Tyr$^{218}$ is located only 2.9 Å away from the C4A atom of the pyrazine portion of FMN. This interaction makes the isoalloxazine ring slightly bent even in the oxidized form as observed from FRP, FRase I, and NOX (21–24).

The ribityl group of FMN makes three hydrogen bonds (Fig. 2B): O2$^+$ with a water molecule, O3$^+$ with the amide nitro of Ser$^{398}$, and O4$^+$ with the Nε atom of Lys$^{167}$. The negative charge of the phosphate is balanced by the positively charged N-1 and N-2 atoms of Arg$^{15}$ and the Nε-2 atom of His$^{11}$, and also forms a hydrogen bond to the amide nitro atom of Ser$^{15}$. Additional water-bridged hydrogen bonds are present with the N-1 and N-2 atoms of Arg$^{15}$, the side chain amide nitro atom of Lys$^{167}$ and the oxygen atom of His$^{11}$, to stabilize of the phosphate.

Comparison of NfsA with FRP—A superimposition of NfsA onto FRP yields an r.m.s.d. for Ca atoms of 0.54 Å for the entire polypeptide chain (Fig. 3A). The superimposition of these two structures reveals that the residues that form the active site pocket except for helices I and J superimpose well on each other (Fig. 4). The well conserved residues are localized on the right side of the active site. Especially Arg$^{15}$, Gln$^{67}$, Arg$^{133}$, Lys$^{167}$, Arg$^{15}$, Ser$^{401}$, and Ser$^{418}$ are conserved at the atomic level of the three-dimensional structure (Fig. 5). Considering the same substrate specificity with regard to the first substrate between NfsA and FRP in the Ping Pong Bi Bi catalytic system, some of these conserved and correspondent residues might be involved in NADPH binding.

There is a subtle, but clear difference around the active site pocket between NfsA and FRP (Fig. 4). The main differences are localized to a particular region on the left side of the active site, that contains residues 192 to 225, including a part of helix I, the following flexible loop and helix J. This region shows an average r.m.s. deviation of 1.8 Å in distance. Several residues are exposed to the active site cleft: Tyr$^{199}$, Tyr$^{200}$, Arg$^{203}$, His$^{215}$, Arg$^{218}$, Thr$^{213}$, Lys$^{222}$, Ser$^{224}$, and Arg$^{227}$ in NfsA, and Tyr$^{199}$, Tyr$^{200}$, Trp$^{212}$, Glu$^{215}$, Val$^{216}$, Lys$^{219}$, Leu$^{220}$, and

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3 Residue marked with B is from the other monomer.
Arg\textsuperscript{225} in FRP. Unconserved residues between NfsA and FRP that form part of the active site are mainly localized on helix J (Fig. 3B). In addition to the rigid-body movement of helix J between both enzymes (Fig. 4), substitutions of amino acids contribute to a more closed active site, especially around position 222, in NfsA relative to FRP (Fig. 4). This observation is consistent with the specificity for the second substrate, in that flavins, more bulky molecules than nitrocompounds, are good substrates for FRP, but poor substrates for NfsA (16, 40). Fig. 4 also shows that the conserved residues on the amino acid alignment between NfsA and FRP are not always conserved in their structures, as observed at position 199 and 200 in NfsA and FRP.

\textbf{Mutational Analysis of NfsA—} To get direct information about the NADPH binding and the catalytic pathway of this enzyme, we tried to provide the crystal of NfsA complexed with NADPH, NADP\textsuperscript{+}, or its analogues by the soaking method. However, crystals soaked with 100 mM NADPH cracked. As moderate concentration (10 mM or less) of NADPH was used as an additive, the protein catalyzed the reduction in the crystal, losing the yellow color from the oxidized form of the cofactor FMN. The diffraction from this crystal was quite poor and that seemed to be attributed to the disorder of the protein symmetry in the crystal. In using NADP\textsuperscript{+} or its analogues, the meaningful electron density could not be found in the difference Fourier map. This suggests that NADPH binding influences the conformation of the loop between helices I and J in the vicinity of the catalytic center FMN, considered that Gly\textsuperscript{204} and Arg\textsuperscript{208} of the loop are involved in the crystal packing interaction.

The solvent exposed loop region between helices I and J is 20 Å away from the catalytic center (isoalloxazine ring) of the cofactor FMN (Fig. 4). However, this loop could be close to the binding site of the adenosine moiety of NADPH bound to the active site of NfsA in an extended conformation. This loop has a flexible conformation as shown by the average \textit{B}-factor of 25.5 Å\textsuperscript{2}. The crystal packing may define the loop conformation in the crystal and it is unclear whether this structure of the loop is the most stable in solution.
To investigate the role of the flexible loop consisting of residues 202 to 211 in the putative NADPH-binding site, two positively charged amino acids, Arg\textsuperscript{203} and Arg\textsuperscript{208}, were chosen for site-directed mutagenesis (Fig. 3B). These two residues were considered to be candidates for NADPH binding in the FRP crystal structure (21). Arg\textsuperscript{203} is one of the completely conserved residues in the active site according to the amino acid sequence alignment among NfsA, NfrA1, and FRP. On the other hand, Arg\textsuperscript{208} in NfsA corresponds to Lys\textsuperscript{208} in FRP and Arg\textsuperscript{217} in NfrA1. It is possible that the side chain of Arg\textsuperscript{208} recognizes NADPH considering the possibility of refolding of the flexible loop in solution, although Arg\textsuperscript{208} is not directed toward the active site in the present structure. As observed in other NADPH binding enzymes, there are examples where positively charged residues, such as arginine, lysine, and histidine, are involved in the recognition of the 2'-phosphate group of NADPH through a hydrogen bond (41–45). Thus, if Arg\textsuperscript{203} or Arg\textsuperscript{208} interact with NADPH, mutation of these residues will contribute to a decrease in NADPH binding affinity to NfsA.

The catalytic activities of whole cell extracts of the mutant and wild type enzymes were measured using NADPH as an electron donor and nitrofurazone as an electron acceptor. As compared with the relative activity of the wild-type enzyme, the R203A mutant showed low activity (Table II). On the other hand, the relative activity of the R208A mutant is similar to that of the wild-type enzyme (Table II). To characterize R203A
NfsA further, kinetic parameters were determined. The $K_m$ value for NADPH of R203A NfsA is 33-fold higher than the $K_m$ of the wild-type, whereas the $K_m$ value for nitrofurazone of R203A enzyme is comparable to that of wild-type (Table III, data of wild-type enzyme were obtained and modified from Ref. 16). These findings suggest that R203A but not R208A is involved in interactions with NADPH, most probably through the 2'-phosphate.

**DISCUSSION**

**Comparison of FMN Binding**—The binding conformation of the cofactor FMN in NfsA is quite similar to that in FRP (21) and several other flavoproteins (23, 24). Comparing FMN binding of NfsA with that of FRP reveals highly conserved hydrogen bonds and hydrophobic interactions that contribute to FMN binding. The main difference between NfsA and FRP in the flavin environment is the replacement of His69 in NfsA with Tyr69 in FRP (Fig. 2B). The O$_{\text{2A}}$ atom of Tyr69 in FRP forms a hydrogen bond to the O2A atom (21), while a water molecule is located within a distance capable of making a hydrogen bond to the O2A atom of the FMN in NfsA. The N-5 nitrogen atom of the FMN is exposed to the solvent, according to a solvent accessibility calculation using a 1.4-Å probe (Fig. 2B). One of two N-1 atoms in the NfsA dimer is completely solvent inaccessible, suggesting that the N-5 atom is the likely candidate for receiving a hydride from the C-4 atom of NADPH in the NADPH oxidation process.

The Specificity of the Second Substrate in the NfsA/FRP Subfamily—NfsA catalyzes poorly the reduction of flavins (16), whereas NfrA1 and FRP have broader electron acceptor specificity and catalyze efficiently the reduction of not only nitrocompounds but also flavins (20). Considering the differences in the active sites between NfsA and FRP suggests that the specificity for the second substrate depends partly on the volume of the active site pocket. Indeed, a previous mutagenesis study indicates that NfsA is able to accommodate FMN as the second substrate by substituting Glu to Gly at position 99 (25). The side chain of Glu$^{99}$ is located at the interface between the two monomers and participates in three hydrogen bonds. The Oe-1 atom forms a hydrogen bond with the N$\epsilon$ atom of Arg$^{133}$ on the $3_{10}$ helix and the Oe-2 atom forms a hydrogen bond with the N$\gamma$-2 atom of Arg$^{133}$ and the N$\gamma$-1 atom of Arg$^{225}$.

**Proposed Binding Mechanism of NADPH to NfsA**—To consider a possible mechanism of NADPH binding, two minimum requirements may be met. One, that the 2'-phosphate group of NADPH interacts with Arg$^{203}$ (see Table II) and, two, that the nicotinamide ring of NADPH is located near the isoalloxazine ring of FMN for hydride ion transfer between the two rings. In addition, in the crystal structures of NAD(P)H-related oxidoreductases with flavin compounds as cofactors (46–48), the nicotinamide ring of NADPH is stacked with the isoalloxazine ring of FMN such that the N1-C4 axis of the nicotinamide ring is approximately parallel to the N5-N10 axis of the isoalloxazine ring. To best fulfill these conditions, we have tried to build a model of the NfsA-NADPH complex using the present crystal structure of NfsA and the conformations of NAD(P)$^+$ observed in protein crystals (49). The parallel arrangement of the nicotinamide N1-C4 axis with the isoalloxazine N5-N10 axis could not be satisfied in the NfsA-NADPH complex model without considerable conformational change of the NADPH and/or NfsA. Indeed, if the nicotine ring is arranged to satisfy the stacking and parallel arrangement, steric hindrance of the ribose connected to the nicotine ring occurs with the ribityl portion of FMN or the region near Phe$^{42}$ of NfsA. The parallel arrangement of the axes of FMN and NADPH, thus, prevents the stacking of the two rings, and consequently interaction of 2'-phosphate group of NADPH with Arg$^{203}$.

**Fig. 5** shows the proposed model of NfsA-NADPH complex, in which the conformation of NADP is taken from that bound to 6-phosphogluconate dehydrogenase (PDB code 1PGO) (43), which possesses the most extended conformation among the NAD(P)$^+$ conformations observed in protein crystal structures (49). As a result, the N1-C4 axis of NADPH inclines $-60^\circ$.
relative to the N5-N10 axis of FMN in the model. The nicotinamide moiety of NADPH interacts with Arg225 and the surrounding residues Arg133 and Asn134 (Fig. 5). The negatively charged pyrophosphate is likely to attract the positively charged Lys167 and Arg199 that are active site residues conserved between the structures of NfsA and FRP, both of which use NADPH specifically as an electron donor. If NfsA undergoes a conformational change by NADPH induced-fit, a complex satisfying the conditions described above could be formed during the catalytic reaction in solution. Thus, the present model may need revising to give a complete picture of the binding mode of NADPH.

Preliminary measured catalytic activities of whole cell extracts of wild-type, R203A, and R208A enzymes using NADH as an electron donor showed that quite low, but similar relative activity to one another (Table II). One possible presumption is that the difference in the activity shown only when using NADPH results from the difference in the structure between NADPH and NADH, and also that the 2′-phosphate peculiar to NADPH interacts with the side chain of Arg203. The observation that R203A mutant has nitroreductase activity when using NADPH suggests that several amino acids might participate in the recognition of the 2′-phosphate group. In this proposed model, the 2′-phosphate is located in the vicinity of a hydrophilic cluster formed by Arg203 and surrounding residues, such as Asp165, Tyr199, and Tyr209 (Fig. 5). These residues are also conserved, except that Gly171 in NfrA1 and Glu165 in FRP corresponding to Asp165 in NfsA.

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FIG. 5. Proposed binding mode of NADPH in the active site of NfsA. An NADPH molecule derived from the crystal structure of 6-phosphogluconate dehydrogenase (43) (Protein Data Bank entry 1PGO) is overlaid on the active site. The putative residues involved in NADPH binding are drawn in a ball and stick model. Each atom is colored according to atom type. Chemical bonds of protein and NADPH are colored in green and those of FMN in yellow. The two monomers of the molecule are colored in dark orange and blue.
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Structure and Site-directed Mutagenesis of a Flavoprotein from Escherichia coli That Reduces Nitrocompounds: ALTERATION OF PYRIDINE NUCLEOTIDE BINDING BY A SINGLE AMINO ACID SUBSTITUTION
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