# NrdH-Redoxin of Corynebacterium ammoniagenes Forms a Domain-Swapped Dimer

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# NrdH-Redoxin of *Corynebacterium ammoniagenes* Forms a Domain-Swapped Dimer

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***ABSTRACT*** **NrdH-redoxins constitute a family of small redox proteins, which contain a conserved CXXC sequence motif, and are characterized by a glutaredoxin-like amino acid sequence but a thioredoxin-like activity profile. Here we report the structure of *Corynebacterium ammoniagenes* NrdH at 2.7 Å resolution, determined by molecular replacement using *E. coli* NrdH as model. The structure is the first example of a domain-swapped dimer from the thioredoxin family. The domain-swapped structure is formed by an inter-chain two-stranded antiparallel β-sheet and is stabilized by electrostatic interactions at the dimer interface. Size exclusion chromatography, and MALDI-ESI experiments revealed however, that the protein exists as a monomer in solution. Similar to *E. coli* NrdH-redoxin and thioredoxin, *C. ammoniagenes* NrdH-redoxin has a wide hydrophobic pocket at the surface that could be involved in binding to thioredoxin reductase. However, the loop between α2 and β3, which is complementary to a crevice in the reductase in the thioredoxin–thioredoxin reductase complex, is the hinge for formation of the swapped dimer in *C. ammoniagenes* NrdH-redoxin. *C. ammoniagenes* NrdH-redoxin has the highly conserved sequence motif W61-S-G-F-R-P-[DE]67 which is unique to the NrdH-redoxins and which determines the orientation of helix α3. An extended hydrogen-bond network, similar to that in *E. coli* NrdH-redoxin, determines the conformation of the loop formed by the conserved motif. Proteins 2004;55:613–619.**


**Key words: glutaredoxin-like proteins, NrdH, 3D domain swapping, crystal structure, thioredoxin fold, redox active disulfide**

## INTRODUCTION

"Glutaredoxin-like proteins," NrdH-redoxins (NrdH), constitute a class of redox active proteins, which are members of the thioredoxin superfamily.[1] NrdH-redoxins have been found in *E. coli* and several other organisms, in particular organisms lacking glutathione.[2,3] NrdH is part of the *nrdHIEF* operon and is the functional hydrogen donor for the NrdEF ribonucleotide reductase.[2–4] *C. ammoniagenes nrdHIE* and *nrdF* are transcribed independently, each one from its own promotor, and the NrdEF ribonucleotide reductase system is essential for aerobic growth of *C. ammoniagenes*.[5] Under microaerophilic conditions, type Ib RNR in combination with NrdH is a much more efficient enzyme than type Ia RNR (NrdAB). It has been suggested that the NrdEF-NrdH system may be the evolutionary oldest RNR reducing system, capable to function in a microaerophilic environment, where no glutathione was yet available.[6]

NrdH-redoxins are small proteins (9–10 kDa) exhibiting sequence identities in the range of 34–99%, and are homologous to glutaredoxins. For instance, *C. ammoniagenes* NrdH has 41% sequence identity to *E. coli* NrdH and 18% identity to glutaredoxin 3.

Characteristics of NrdH-redoxins are the common fold and a conserved active site motif C-V-Q-C or C-M-Q-C that contains the redox-active cysteine residues. The pattern of residues linking the two cysteine-residues is thus different from thioredoxins and glutaredoxins, -C-G-P-C- for thioredoxin and -C-P-Y-C- for most glutaredoxins.[7–9] NrdH-redoxins also contain a highly conserved sequence motif [WF]-S-G-F-[RQ]-P-[DE] that is unique for this class.[6] The motif is part of a loop that connects strand β3 to helix α3, and is stabilized by an extensive hydrogen-bond network.

These redoxins are structurally most similar to glutaredoxins, which are specifically reduced by glutathione (GSH), although NrdH-redoxins, like thioredoxins, depend on thioredoxin reductase (TrxR) for reduction. *E. coli* NrdH contains a deep hydrophobic pocket at the surface, similar to that observed in thioredoxin, and modelling studies[6] suggest that *E. coli* NrdH binds to TrxR in a similar way as thioredoxin in the Trx–TrxR complex.[10]

We have solved the crystal structure of *C. ammoniagenes* NrdH to 2.7 Å by molecular replacement, using the

*Abbreviations:* NrdH, NrdH-redoxin; GSH, glutathione; Trx, thioredoxin; Grx, glutaredoxin; Grx1, *E. coli* glutaredoxin-1; Grx3, *E. coli* glutaredoxin-3; TrxR, thioredoxin reductase; RNR, ribonucleotide reductase.

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*E. coli* NrdH structure[6] as a search model. The crystal structure reveals an unexpected three-dimensional domain-swapping. This is the first time that a domain-swapped form of a protein from the thioredoxin-family is reported.

## MATERIALS AND METHODS

### Sample Preparation

The vector containing the gene for *C. ammoniagenes* NrdH (GenBank Accession Nr. Y09572)[11] was transformed into BL21-CodonPlus (Stratagene) and the cells were grown as described.[2] Frozen cells were suspended in 10 volumes of 50 mM Tris-HCl pH 7.5, 1 mM EDTA and 0.2 mg/ml lysozyme, and disrupted by sonication. The cleared raw extract was dialyzed overnight against 20 mM Tris-HCl, 1 mM EDTA, pH 9.5, and applied to a XK 26/20 column packed with 70 ml DEAE cellulose (Pharmacia), equilibrated with the same buffer. The protein was eluted with 650 ml 50 mM Tris-HCl, 1 mM EDTA, 500 mM NaCl, pH 7.5. The NrdH containing fractions were pooled and applied to a XK 26/20 column packed with G50 resin (Pharmacia), equilibrated with 50 mM Tris-HCl, 1 mM EDTA, 500 mM NaCl, 10% glycerol, pH 8.0. NrdH containing fractions were pooled and dialyzed against 20 mM Tris-HCl, 1 mM EDTA, pH 9.5 and applied to a Poros 20 HQ column (5 ml), (Applied Biosystems), equilibrated with 20 mM Tris-HCl, pH 7.5. The protein was eluted by a NaCl gradient. NrdH containing fractions were pooled and concentrated using a 3 K concentrator (Amicon) after the addition of 50 mM Tris-HCl, pH 8.0, 500 mM NaCl and 10% glycerol. The protein was stored at −20°C. The yield was 80 mg pure NrdH protein from 6 g of cells.

### Protein Analysis

The homogeneity of the purified protein was analysed with SDS gel electrophoresis.[12] The protein concentration was determined with Coomassie Plus Protein Assay Reagent (Pierce). The identity of *C. ammoniagenes* NrdH was confirmed by mass spectrometry at the Protein Analysis Centre, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm.

### Size Exclusion Chromatography

Molecular weight determination was performed by size exclusion chromatography on a FPLC-Superdex column (Pharmacia) at several different pH-values with the following buffers: 50 mM sodium phosphate buffer, 200 mM NaCl, pH 5.0; 20 mM Sodium phosphate buffer 125 mM NaCl, pH 6.0; 20 mM Tris/HCl, 200 mM NaCl, with addition of 1.) 1 mM DTT, 2.) 0.1% octyl βD-glucopyranoside; 50 mM Bis/Tris, 200 mM NaCl, 0.1% octyl β-D-glucopyranoside, pH 9.0; 50 mM Tris/HCl, 300 mM NaCl, 10% glycerol, pH 8.2. The column was calibrated with HSA (67 kDa), albumin (45 kDa), myoglobin (17.6 kDa), ribonuclease (13.1 kDa) *E. coli* NrdH (9.1 kDa) and vitamin B12 (1.3 kDa). For each molecular weight determination 100 μl of protein (0.5 mg/ml) were used.

### Reduction of Insulin Disulphides

The experiments were carried out as described by Holmgren et al., 1979[12] and Jordan et al., 1997,[2] with slight modifications. The reaction was followed as the increase in absorbance at 600 nm due to the precipitation of insulin when reduced to A and B chains.

**TABLE I. Data Collection and Refinement Statistics**

| | | |
|---|---|---|
| Data collection | | |
| Wavelength (Å) | 1.5418 | |
| Resolution (Å) | 25.0–2.70 | (2.75–2.70) |
| $R_{sym}$ (%) | 7.9 | 39.7 |
| $\langle I \rangle / \langle \sigma(I) \rangle$ | 11.7 | 3.7 |
| Completeness (%) | 98.6 | 98.1 |
| Total number of reflections used | 20,347 | |
| Unique reflections | 5187 | |
| Refinement statistics | | |
| Reflections (number) | 5179 | |
| Test set | 237 | |
| Resolution (Å) | 23.7–2.70 | (2.76–2.70) |
| Number of protein atoms | 2292 | |
| Number of water molecules | 37 | |
| $R_{free}$ (%) | 28.7 | (42.2) |
| $R_{cryst}$ (%) | 24.6 | (32.4) |
| B factor (Å$^2$) Protein atoms | 32.4 | |
| B factor (Å$^2$) Water molecules | 23.3 | |
| R.M.S. deviations from ideality | | |
| Bond lengths (Å) | 0.006 | |
| Bond angles (°) | 0.89 | |
| Ramachandran plot, residues in most favorable regions (%) | 92.3 | |
| Additional allowed regions (%) | 7.7 | |

### Crystallization and Data Collection

Crystals of *C. ammoniagenes* NrdH were obtained by vapor diffusion. The protein solution was dialyzed against 0.05 M CHES-buffer, pH 9.14 and concentrated to 25 mg/ml. Three (3) μl of protein solution was mixed with 3 μl mother liquor (1.28 M Na-citrate, 88 mM Tris/HCl, pH 7.86, 4.4 % MPD) and 1 μl 0.34 mM spermidine. Hanging drops were equilibrated against the mother liquor at 20°C and hexagonal crystals, up to 1 mm in length and 0.2 mm in diameter, were obtained after 1–2 weeks.

A native diffraction data set was collected at 100K on a Rigaku rotating anode with a MAR Research imaging plate. Before transfer into the nitrogen gas stream, the crystals were soaked for a few seconds in a solution of 69.3 mM Tris/HCl, pH 7.9, 1.0 M Na-citrate, 17.3% glycerol, 0.045 M spermidine, 3.5% MPD. The data were integrated with DENZO and scaled with SCALEPACK[13] and further processing was done within the CCP4 suite of programs[14] (Table I). The crystals belong to space group $P6_3$ with cell dimensions of a = 89.64 Å, b = 89.64 Å, c = 40.15 Å and contain two molecules per asymmetric unit.

### Phasing, Model-Building, and Refinement

The structure was solved by molecular replacement using the program AMORE[15] with the NrdH-redoxin from *E. coli*[6] (PDB accession: 1H75) as search model. Refinement was performed with the use of the CNS program package.[16] This refinement consisted of anisotropic scaling, bulk-solvent correction, simulated annealing, conjugate gradient minimization and isotropic B-factor refine-

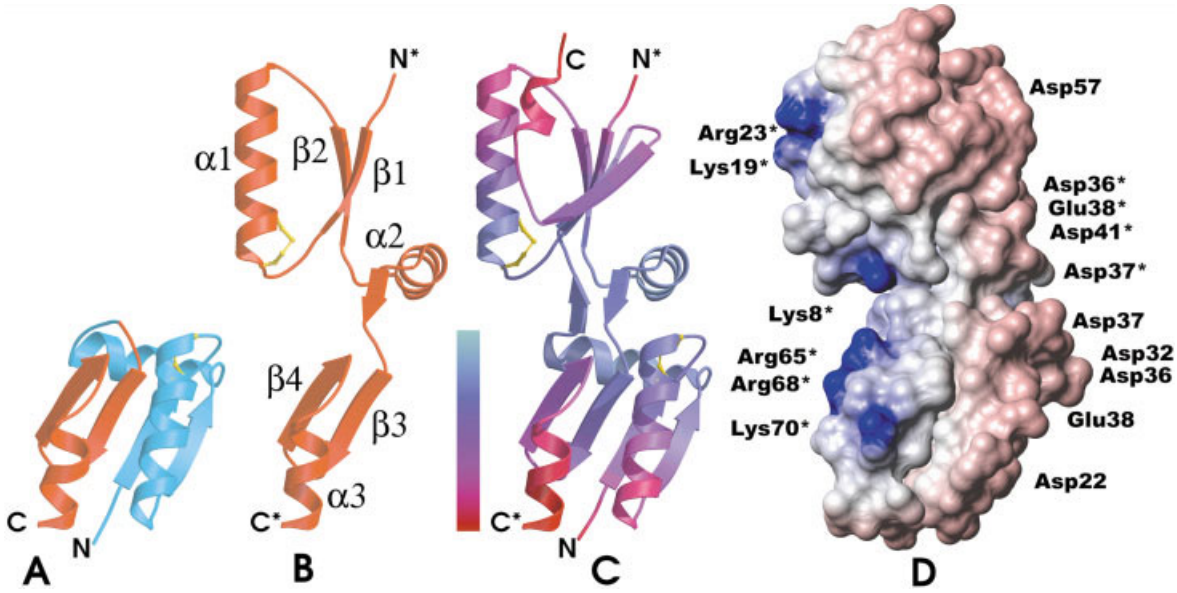


Fig. 1. Schematic drawings of the three-dimensional structure of *C. ammoniagenes* NrdH. The nomenclature of Martin (1995)[7] was used for the helices and sheets and does not include the β-sheet of the dimer-interface. The redox active cysteine residues at the N-terminal of helix α1 are shown as ball-and-stick models. **A**: Closed monomer. **B**: Open monomer. **C**: Domain-swapped dimer, colored according to the B-factors from light blue (20 Å$^2$) to red (45 Å$^2$) as indicated on the reference bar. **D**: surface representation of the domain-swapped dimer. The surface was calculated with program MolMol[22] using a solvent radius of 1.4 Å and colored according to the electrostatic potential, calculated with the program's "simplecharge" option.

ment against the maximum likelihood target. Tight non-crystallographic symmetry restraints were used whenever possible to improve the data to parameter ratio.

After some rounds of refinement it was realized that the structure was wrong around residue 49. Calculation of a simulated annealing omit-map showed clearly that the error was due to domain-swapping at this point. After re-building the chain and continuing refinement the R-factors dropped and the maps looked excellent also in this area. The values for $R_{cryst}$ and $R_{free}$ after refinement were 24.3% and 28.7%, respectively, with the test set used for $R_{free}$ calculation containing 4.6% of the reflections. Model geometry was analyzed with PROCHECK.[17] Further details of the refinement statistics are given in Table I.

In all figures the A-chain of the "open" monomer (domain-swapped)[18,19] is marked with an asterisk (*). We use a model of "closed" (unswapped) monomeric *C. ammoniagenes* NrdH for all structure comparisons. This model was constructed by simply reconnecting the chains of the domain-swapped dimer at residue 49 and performing some energy minimization. Minimal structural perturbations are introduced except at the swap site. Structure comparisons and structure alignments were carried out with the programs TOP and MAPS using default parameters.[20,21] Figures were produced using the programs MolMol,[22] O,[23] Molscript[24] and Raster3D.[25] The coordinates and structure factors have been submitted to the Protein Data Bank, accession code 1R7H.

## RESULTS

### Protein Characteristics and Functional assay

*C. ammoniagenes* NrdH was expressed as soluble, active and monomeric protein in *E. coli* BL21-CodonPlus. The molecular weight of the protein obtained by MALDI-ESI was 8.15 kDa in excellent agreement with the theoretical value, 8.159 kDa (the N-terminal methionine is cleaved off). The protein showed a single band (8 kDa) on a SDS-PAGE gel. On a Superdex 75 column the protein eluted as single, symmetrical peak with an apparent weight of 10 kDa. The apparent molecular weight was ca 2 kDa higher than expected, a result of the interaction of NrdH with the matrix.

The expressed *C. ammoniagenes* NrdH is a potent reductant of insulin disulphides in the presence of DTT similarly to *E. coli* NrdH.[2]

### Overall Structure of the Domain-Swapped Dimer

The crystal structure reveals an unexpected dimeric structure of NrdH which is due to three-dimensional domain-swapping (Fig. 1). The refined model of the NrdH dimer at 2.7 Å resolution contains residues 1–74 from both monomers in the asymmetric unit and in total 37 water molecules. Domain-swapping occurs between α2 and β3 of the subunit. An inter-chain two-stranded anti-parallel β-sheet is formed from Tyr48 to Gln50 of both monomers at the dimer interface. The Gln50 side chains form weak hydrogen bonds with Ser62 of the same monomer. Helix α2 of both molecules pack against each other, stabilized by electrostatic interactions: Arg40* and Asp37* interact with Asp41 and Arg40 of the other subunit [Fig. 2(A)]. The overall B-factors in the core of the domain-swapped dimer is significantly lower than in the outer parts of the protein [Fig. 1(C)], indicating less flexibility in the area around the domain-swapped interface.

The NrdH dimer forms an elongated structure with dimensions of 20 × 50 Å and is a bipolar charged molecule [Fig. 1(D)]. One side of the molecule has an overall positive

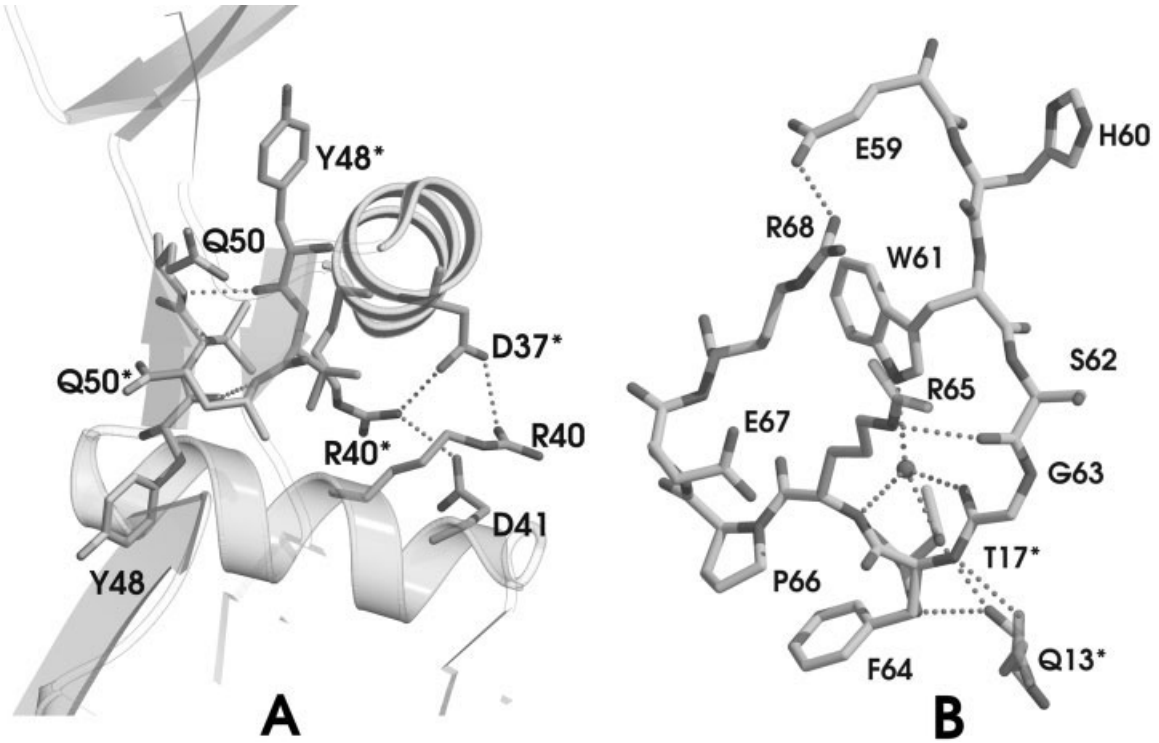


Fig. 2. Hydrogen-bond networks in the domain-swapped NrdH dimer. **A**: The dimer interface. The β-sheet of the interface is formed by the residues 48–50 of both domain-swapped monomers. The residues D37*, R40*, D40, and D41 of both α2 helices form a hydrogen-bond network. The molecule has the same orientation as in Figure 1(A–D). **B**: View of the hydrogen-bond network in the structure of *C. ammonigenes* NrdH formed by the highly conserved WSGRFP sequence motif. Hydrogen bonds are drawn as dashed lines.

**TABLE II. Structural Similarities of NrdH Redoxin With Other Redoxins in the Thioredoxin Family**

| Molecule | Structurally equivalent residues | Sequence identical residues | R.M.S.D. (Å) |
|---|---|---|---|
| *E. coli* NrdH (1H75) | 72 | 30 | 0.83 |
| *E. coli* Glutaredoxin 3 (1FOV) | 64 | 12 | 1.37 |
| *A. thaliana* glutathione S-transferase (1GNW) | 59 | 9 | 1.31 |
| T4 glutaredoxin (1AAZ) | 56 | 15 | 1.42 |
| *E. coli* Glutaredoxin 2, 1–72 (1G7O) | 59 | 9 | 1.49 |
| *M. jannaschii* Mj0307 (1FO5) | 37 | 4 | 1.76 |

charge (Lys8*, Lys19*, Arg23*, Arg65*, Arg68*, Lys70*), whereas the other side has an overall negative charge (Asp 22, Asp32, Asp36, Asp36*, Asp37, Asp 37*, Glu38, Glu38* Asp41*, Asp57).

## Overall Structure of the Model of the Closed Monomer

Eisenberg has defined a monomeric, unswapped polypeptide as a "closed" monomer and the conformation of the corresponding domain in the swapped oligomer as the "open" monomer.[18,19] The closed monomer of *C. ammoniagenes* NrdH belongs to the thioredoxin superfamily and shares the thioredoxin fold with the highly conserved *cis*-proline (P52) of the thioredoxin family at the beginning of strand β3. The model of the *C. ammoniagenes* NrdH closed monomer is structurally most similar to *E. coli* NrdH[6] (Table II). An alignment gives an root mean square (RMS) of 0.83 Å for 72 Cα-atoms, and both proteins share features that are typical for the NrdH subclass of redoxin proteins.[6]

*C. ammoniagenes* NrdH contains the NrdH-redoxin typical W61-S-G-F-[RQ]-P-[DE]67 motif, which forms the end of strand β4, the following loop and the beginning of helix α3. A network of hydrogen bonds positions helix α3 relative to the remaining part of the molecule [Fig. 2(B)]. As in *E. coli* NrdH, a water molecule, which forms hydrogen bonds to atom Nϵ1 of W61, Oγ1 of T17, and main chain oxygen and nitrogen atoms of G63 of R65, respectively, plays a central role in this network. A second water molecule that interacts with the main chain oxygen of residue G63 and NH1 of R65 in *E. coli* NrdH is missing; instead the main chain oxygen of S62 interacts with NH1of R65 in *C. ammoniagenes* NrdH. A hydrogen bond between

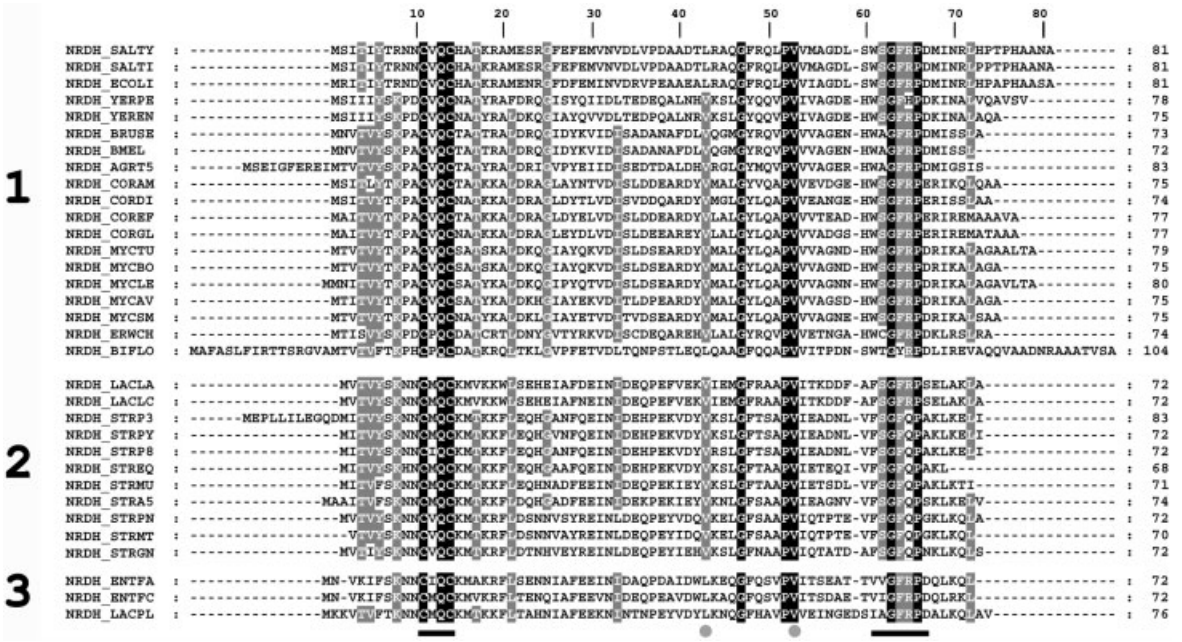


Fig. 3. The NrdH protein family, sequence alignment of NrdH-redoxins. The numbering refers to *E. coli* NrdH. Invariant residues are shaded black. Residues with 70% identity are shaded grey. Residues forming the bottom of the hydrophobic pocket in *E. coli* and *C. ammoniagenes* NrdH are marked with a grey circle. NrdH-typical motifs are marked with an black bar. Abbrevations: NRDH_SALTY, *Salmonella typhimurium*; NRDH_SALTI, *Salmonella typhi*; NRDH_ECOLI, *Escherichia coli*; NRDH_YERPE, *Yersinia pestis*; NRDH_YEREN, *Yersinia enterocolitica*; NRDH_BRUSE, *Brucella suis*; NRDH_BMEL, *Brucella melitensis*; NRDH_AGRT5, *Agrobacterium tumefaciens*; NRDH_CORAM, *Corynebacterium ammoniagenes*; NRDH_CORDI, *Corynebacterium diphtheriae*; NRDH_COREF, *Corynebacterium efficiens*; NRDH_CORGL, *Corynebacterium glutamicum*; NRDH_MYCTU, *Mycobacterium tuberculosis*; NRDH_MYCBO, *Mycobacterium bovis*; NRDH_MYCLE, *Mycobacterium leprae*; NRDH_MYCAV, *Mycobacterium avium*; NRDH_MYCSM, *Mycobacterium smegmatis*; NRDH_ERWCH, *Erwinia chrysanthemi*; NRDH_BIFLO, *Bifidobacterium longum*; NRDH_LACLA, *Lactococcus lactis*; NRDH_LACLC, *Lactococcus cremoris*; NRDH_STRP3, *Streptococcus pyogenes* (serotype M3); NRDH_STRPY, *Streptococcus pyogenes*; NRDH_STRP8, *Streptococcus pyogenes* (serotype M18), NRDH_STREQ, *Streptococcus equi*; NRDH_STRMU, *Streptococcus mutans*; NRDH-_STRA5, *Streptococcus agalactiae* (serotype V); NRDH_STRPN, *Streptococcus pneumoniae*; NRDH_STRMT, *Streptococcus mitis*; NrdH_STRGN, *Streptococcus gordonii*; NRDH_ENTFA, *Enterococcus faecalis*; NRDH_ENTFC, *Enterococcus faecium*; NRDH_LACPL, *Lactobacillus plantarum*. The sequences were obtained from SwissProt, TrEMBL, and Entrez genomes. (http://www.ncbi.nlm.nih.gov/genomes/static/eub_g.html). The alignment was done with the program Clustal[34] and shaded with GeneDoc.[35]

NH1 of R68 and Glu59 Oϵ2 replaces the salt bridge between E67 and R65 in *E. coli* NrdH.[6] As in *E. coli* NrdH, the main-chain oxygen atom of Q13 and the nitrogen of T17 form a hydrogen bond [Fig. 1(B)].

### The Active Site

The reactive cysteines are located at the beginning of helix α1. The electron density map clearly shows formation of a disulfide bond between residues C11 and C14, and the structure of NrdH described here is thus the oxidized protein. The architecture of the active site disulfide of the *C. ammoniagenes* and *E. coli* NrdH molecules are nearly identical. While the first cysteine of the active site, C11, is surface accessible, C14 lies in a groove formed by residues R8, C11 and Q13.

### Modelling of Interactions With Thioredoxin Reductase

The *C. ammoniagenes* NrdH monomer has a hydrophobic pocket next to the active site, suggested to be important in the interactions with TrxR.[6] V43 forms the bottom of this cavity, surrounded by the hydrophobic residues Y6, I33, A51, and V53. The architecture is only slightly different from that in the *E. coli* protein where the bottom of the hydrophobic pocket is formed by L43.[6] The hydrophobicity of the side chains lining the pocket is conserved among all known NrdH proteins (Fig. 3), suggesting a similar structural design of the pocket in all NrdH redoxins. However, NrdH from *C. ammoniagenes* lacks the strongly positive charged surface around the suggested Trx binding cleft present in *E. coli* NrdH. Superposition of the model of the closed monomer of *C. ammoniagenes* NrdH onto *E. coli* NrdH in the model of the *E. coli* NrdH-TrxR complex,[6] indicates that F141 and F142 of TrxR from *E. coli*, which are conserved in TrxR from *C. ammoniagenes*, fit well into the hydrophobic pocket of *C. ammoniagenes* NrdH, similarly to the interactions in the *E. coli* NrdH-TrxR model complex.

The loop from helix α2 to strand β3 in Trx occupies a complementary groove on the surface of TrxR[10] in the complex and the corresponding loop in *E. coli* NrdH could be fitted into this groove in the model complex.[6] In *C. ammoniagenes* NrdH however, this loop is the hinge involved in domain swapping and modelling of this interaction could thus not be performed. Furthermore, in the dimer structure of *C. ammoniagenes* NrdH, the hydrophobic pocket described above is completely shielded by the second subunit and formation of the domain-swapped dimer would thus prevent binding and reduction by TrxR.

### Comparison of NrdH Proteins

NrdH proteins are mainly characterized by their size (< 10 kDa), and three sequence motifs: the active site C-[VM]-Q-C, the conserved *cis*-P52-V53 and the C-terminal [WF]-S-G-F-[RQ]-P-[DE]-motif.

To date (6/6/2003) 26 sequences are annotated as NrdH proteins in Swiss-Prot and TrEMBL databases. A TBLASTN search with the sequence of *C. ammoniagenes*, NrdH against the finished and unfinished genomes at NCBI, found 13 putative NrdH proteins (http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi).

The NrdH sequences show identities of 35–99%, align without gaps, and contain the three NrdH motifs (Fig. 3). Based on the sequence differences of the active site and the C-terminal-motif the redoxins form three groups: Group 1 (C-V-Q-C; W-S-G-F-R-P-[ED]), group 2 (C-[MVI]-Q-C; F-S-G-F-[RQ]-P), and group 3 (C-[MI]-Q-C; G-P-X-P). V/L43 and V53 that form the bottom of the hydrophobic pocket where TrxR is suggested to bind, are conserved in all NrdH proteins (Fig. 3).

All found NrdH redoxin sequences are part of the *nrdHIEF* operon, coding for an Ib ribonucleotide reductase system. Species of the genus *Streptococcus* and *Lactobacillus* lack the *nrdI* gene in the operon but it is found elsewhere in the genome[5] (Fig. 4). The gene *NrdF* of *Mycobacterium* and *Corynebacterium* is separated from *nrdE* in a range from 862–3100 bp.[5,26]

## DISCUSSION

Under certain conditions e.g., high local concentration and a destabilizing milieu, almost any protein is capable of 3D domain-swapping.[18] Although the number of known structures of domain-swapped proteins increases, the physiological relevance remains uncertain. Several domain-swapped oligomers are only obtained under non-physiological conditions, e.g., during crystallization experiments, but exist in vivo as monomers.[19,27,28] Domain swapping can not be predicted from the protein sequence but some features, like the formation of anti-parallel β-sheets or presence of glutamine or proline residues in the area of a dimer interface, may promote domain swapping.[18,19,28,29]

The structure of NrdH-redoxin from *C. ammoniagenes* is the first example of domain swapping in the thioredoxin family even though a large number of structures from this protein family has been reported. NrdH of *C. ammoniagenes* shows several similarities to other domain-swapped proteins, making it an interesting target for studies of oligomer formation. The domain-swapped dimer of *C. ammoniagenes* NrdH has formed an anti-parallel β-sheet, similar as in the domain-swapped human prion protein.[28] The pronounced bipolar charge [Fig. 1(D)] is similar as in the domain-swapped form of T4 endonuclease.[30] As in *C. ammoniagenes* NrdH, the domain-swapped RNase A and the diphtheria toxin dimer[31,32] show a gradual decrease of the B-values from the outside of the protein to the monomer–monomer interface [Fig. 1(C)]. This feature may be a common characteristic for domain-swapped proteins, indicating a thermodynamically favorable state.

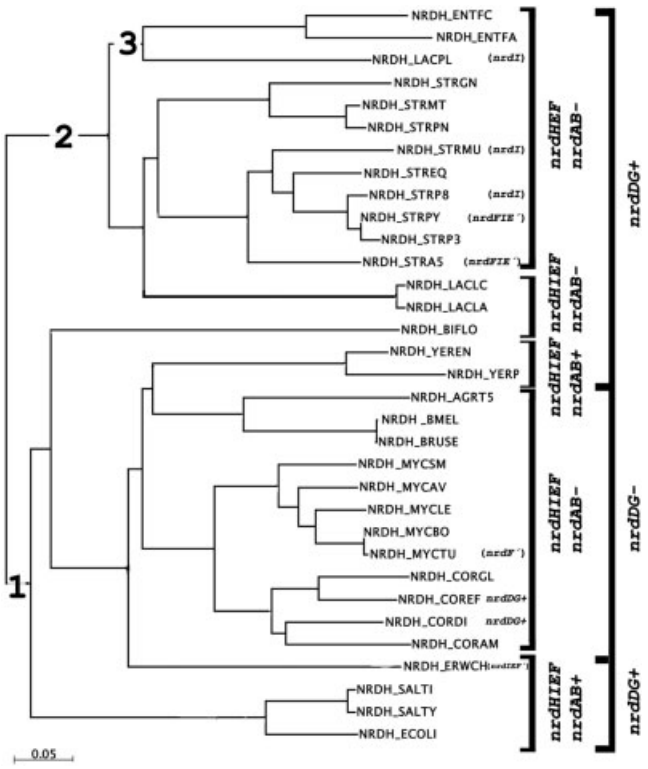


Fig. 4. Phylogenetic tree of the NrdH-protein family and distribution and organization of RNR genes. The genetic organization of *nrdHIEF* and genes is shown on the right. The numbers show the three groups of NrdH proteins as given in Figure 3. The presence or absence of an anaerobic, *nrdAB* and anaerobic RNR system, *nrdDG* is indicated with (+) or (−). The intergenic region between *nrdE* and *nrdF* that is present in species of the genus *Mycobacterium* and *Corynebacterium*[5,26] is not depicted. *Nrd* genes located elsewhere in the genome are put in parenthesis. The presence and location of *nrdHIEF*, *nrdAB*, *nrdDG* genes in the genomes were identified with a BLAST search against GenBank sequences or a TBLASTN search against the Entrez finished and unfinished genomes using the *E. coli* and *C. ammoniagenes* sequences as templates (http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi). The tree was done with NJPlot.[36]

Why is NrdH from *C. ammoniagenes* more disposed to domain swapping than NrdH from *E. coli*? The structures are virtually identical except in the hinge loop that gives rise to domain swapping and in the new dimer interface. The structural differences in the hinge loop are localized to residues 48–50. In the *E. coli* protein these residues, Phe48, Arg49, and Gln50 are part of a bend, making no hydrogen bonds, and Arg49 has a somewhat strained conformation. In the swapped dimer of the *C. ammoniagenes* protein these residues, Tyr48, Val49, and Gln50, have anti-parallel β-sheet conformation with two hydrogen-bonds but with Tyr48 in a slightly strained conformation. It seems that introduction of a valine instead of arginine has favored the β-conformation and that the hydrogen bonds introduced contribute to compensate for the entropy loss upon dimer formation. Another contributing factor is that the dimer surface in the domain-swapped *C. ammoniagenes* NrdH is stabilized by a hydrogen-bond network and electrostatic interactions at the interface involving residues on the α2-helices [Fig. 2(A)].

The model of the closed monomer of *C. ammoniagenes* NrdH is very similar to *E. coli* NrdH. Both molecules share a hydrogen network stabilizing the loop connecting strand β4 to α3 and a characteristic hydrophobic pocket, however, the surface charge around the pocket is different. Modelling studies shows that the *C. ammoniagenes* NrdH closed monomer most probably can bind to thioredoxin reductase in a similar way as modelled for *E. coli* NrdH.[6]

NrdH-redoxin from *C. ammoniagenes* exists as a monomer in solution. However, if domain-swapped NrdH also occurs *in vivo* it could be a mechanism for regulation of its oxidation state since a result of formation of the swapped dimer is the obliteration of the binding site for TrxR, preventing the reduction of NrdH.

The in vivo function of NrdH is still not understood. Experimental data suggest that NrdH is the reductant of class Ib RNR which is supported by the finding that all known NrdH sequences are part of the *nrdHIEF* operon. It was proposed that the NrdHIEF system may be an evolutionary old system, used under microaerophilic conditions or at low cellular glutathione level.[6] Lactic acid bacteria, mycobacteria, and corynebacteria lack the NrdAB genes that code for an aerobic RNR, having the Ib system instead. NrdHIEF is the only RNR system of mycobacteria (Fig. 4) and NrdEF seems to be the major aerobic ribonucleotide reductase system of *Mycobacterium tuberculosis and C. ammonigenes.*[5,33] This indicates an essential role of NrdHIEF for the ribonucleotide metabolism.

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