The Mo-Se active site of nicotinate dehydrogenase

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Nicotinate dehydrogenase (NDH) from Eubacterium barkeri is a molybdenoenzyme catalyzing the hydroxylation of nicotinate to 6-hydroxynicotinate. Reactivity of NDH critically depends on the presence of labile (nonselenocysteine) selenium with an as-yet-unidentified form and function. We have determined the crystal structure of NDH and analyzed its active site by multiple wavelength anomalous dispersion methods. We show that selenium is bound as a terminal Mo–Se ligand to molybdenum and that it occupies the position of the terminal sulfido ligand in other molybdenum hydrolases. The role of selenium in catalysis has been assessed by model calculations, which indicate an acceleration of the critical hydride transfer from the substrate to the selenido ligand in the course of substrate hydroxylation when compared with an active site containing a sulfido ligand. The MoO(OH)Se active site of NDH shows a novel type of utilization and reactivity of selenium in nature.

Eubacterium barkeri | hydroxylase | molybdoprotein | molybdenopterin | selenium

Selenium is an essential component of several enzymes and has a key role in various biological redox processes. Usually selenium occurs in proteins as selenocysteine, which is cotranslationally inserted as the 21st amino acid (1) and is found in a variety of proteins in all 3 kingdoms of life (2). Selenium also finds a natural use as 5-methylaminomethyl-2-selenouridine in the “wobble” position of some tRNAs (3). The ion radii and electronegativities of selenium and sulfur are similar, but selenide is a stronger reducing agent than sulfide. Because of the lower pKas value of selenols compared with thiols selenocysteine is deprotonated under physiological conditions, whereas cysteine is mostly protonated (4). The ionization state together with computational studies demonstrating the catalytic activity of NDH have shown to contain selenium (5), and a recent comprehensive genomic analysis has revealed a clear relationship between selenium and molybdenum utilization across all 3 domains of life (6). Selenium is found as selenocysteine in some prokaryotic molybdenum-containing oxic oxidoreductases like formate dehydrogenase H from Escherichia coli, where it coordinates molybdenum in the oxidized state of the enzyme (7–9). Selenium is a common component of other enzymes, notably members of the molybdenum hydroxylases. While in an earlier report on the structure the presence of selenium in the active site was suggested (25), a latter study using analytical multiple wavelength anomalous dispersion methods showed that the active site does not contain selenium but a linearly-coordinated Cu(I) ion bridged by a μ-sulfido ligand to the molybdenum (28). This μ-sulfido bridge occupies the position of the Mo–Se seen in other molybdenum hydrolases.

The anaerobic soil bacterium E. barkeri is able to ferment nicotinate to propionate, acetate, carbon dioxide, and ammonia with the gain of 1 mol of ATP per mol of nicotinate. The fermentation of nicotinate is initiated by its hydroxylation to 6-hydroxynicotinate catalyzed by NDH (12). NDH has a (αβγδ)2 subunit structure and contains [2Fe-2S] clusters, FAD and a molybdenum center with a pyranopterin cofactor that has been elaborated as the dinucleotide of cytosine and termed molybdopterin cytosine dinucleotide (MCD) (18, 29). The genes encoding NDH occur in the transcriptional order ndhFSLM and are part of a 23.3-kb gene cluster dedicated to the fermentation of nicotinate (30). The NdhF subunit (33 kDa) carries 1 FAD molecule and the NdhS subunit (23 kDa) contains 2 [2Fe-2S] clusters. Contrary to all structurally characterized hydroxylases the molybdenum cofactor appeared to be contained not in 1 but 2 subunits: the NdhL subunit (50 kDa) and NdhM subunit (37 kDa). The most remarkable feature of NDHs is the presence of labile (nonselenocysteine) selenium (14), which is essential to catalyze the hydroxylation reaction (18). Here, we report the X-ray crystal structure of NDH and its Se-containing active site, together with computational studies demonstrating the catalytic profit of the natural selection of Se over its congeners S and O.

Results

Overall Structure. NDH was crystallized by vapor diffusion methods under anoxic conditions in an atmosphere of 95% N2/5% H2. Crystals belonged to the space group P21 and contained the complete dimer of heterotetramers in 1 asymmetric unit. The structure of NDH was determined with Patterson search techniques by using a substructure of 4-hydroxybenzyl-CoA reductase (23) as search model. The final model contains all residues and was refined to 2.2 Å resolution (Table 1).

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The authors declare no conflict of interest.

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Data deposition: The atomic coordinates have been deposited in the Protein Data Bank, www.pdb.org (PDB ID codes 3HRD).

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NDH is a dimer of heterotetramers with (FSLM)₂ subunit composition in solution (12) and all subunits are present in the crystal structure (Fig. 1). The dimer has overall dimensions of 148 × 100 × 70 Å³. The F subunit (296 residues) harbors the FAD cofactor, which interacts with the N terminal (residues 1–57) and middle domain (residues 58–178) through its adenosin and ribityl moiety. The C-terminal domain (residues 179–291) only interacts with FAD through K187F, whose amino group is far from the NAD⁺/NADP⁺ access to the N5 position is usually blocked by a tyrosine (carbon monoxide dehydrogenase from *Oligotropha carboxydivorans*, quinoline 2-oxidoreductase) or tryptophan side chain (carbon monoxide dehydrogenase from *Hydrogenophaga pseudoflava*). In contrast, the less bulky side chain of isoleucine is found in the NAD⁺/NADP⁺-reducing NDH and bovine and bacterial XOR (21, 22). All 3 domains of the F subunit show a mixed α/β-fold. The S subunit (157 residues) comprises 2 domains, each coordinating 1 [2Fe-2S] cluster. The N-terminal domain (residues 1–79) is similar to “plant-type ferredoxins” and binds the [2Fe-2S] cluster closest to the FAD, which is designated as Fe/S II or type II on the basis of its EPR characteristics in homologous molybdenum hydroxylases (31–33). The C-terminal domain (residues 80–157) displays a 4-helix bundle with 2-fold symmetry unique to molybdenum hydroxylases (19) and coordinates the [2Fe-2S] cluster (type I) closest to the molybdopterin. The L subunit (425 residues) and M subunit (330 residues) harbor the Mo-pyranopterin cofactor. Both subunits have an extended structure and lie approximately perpendicular on top of each other. The L subunit interacts with the M and S subunits and has an N-terminal extension (residues 1–28₁) that wraps around the C-terminal domain of the S subunit. A middle domain (residues 29₁–129₁ and 181–277₁) with 2 antiparallel β-sheets of 2 and 7 strands and 3 α-helices follows the N-terminal extension. The C-terminal domain of the L subunit is dominated by a mixed 5-stranded β-sheet flanked on 1 side by 2 α-helices that continue into a 2-stranded antiparallel β-sheet and a C-terminal α-helix. The M subunit can be divided into 2 domains, both containing mixed 4-stranded β-sheets and 3 α-helices (Fig. 1).

Both monomers of NDH show the same arrangement of subunits and cofactors building 2 independently working electron transfer chains (Fig. 1), similar to other structurally characterized molybdenum hydroxylases. However, NDH is unusual in containing 4 subunits per monomer, whereas other molybdenum hydroxylases of known structure, contain 1, 2, or 3 subunits. Compared with all structurally characterized molybdenum hydroxylases, the Mo-pyranopterin binding subunit/domain has

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**Table 1. Statistics on diffraction data and structure refinement**

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In the datasets at the Se edge the values given are for unmerged Friedel mates. The values in parentheses indicate the highest resolution.

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**Fig. 1.** Overall structure of NDH. Ribbon plot representation of the NDH dimer. In the left monomer each subunit has its own color with green and red for the MCD coordinating L and M subunits, blue for the [2Fe-2S] clusters containing S subunit, and yellow for the FAD containing F subunit. The right monomer is colored in different shades of gray. Cofactors are labeled, and FeS₁ and FeS₂ indicate the position of the type I and type II [2Fe-2S] clusters of the S subunit. The shortest distances between the cofactors are 5.4 Å (MCD-FeS₁), 11.5 Å (FeS₁-FeS₂), and 6.4 Å (FeS₂-FAD), with the shortest metal-to-metal distances of 14.7 Å (MCD-FeS₁) and 12.4 Å (FeS₂-FeS₃). All pictures were prepared by using PyMol (48).
been split into 2 polypeptides in NDH: the L subunit corresponds to the N-terminal domain and the M subunit is homologous to the C-terminal domain. Bioinformatic analysis has revealed split molybdopterin subunits in 1 of 3 other bacterial NDHs (SI Text and Fig. S1). *Moorella thermoacetica, Carboxythermus hydrogenoformans, Petrogentota mobilis, Clostridium asparagiforme,* 5α-proteobacterial species, *Alkaliphilus oremlandii,* and *Alkaliphilus metalliredigens* have split subunits in NDH homologs of *hitherto unknown catalytic activity (SI Text and Fig. S2).*

### Active-Site Structure and Selenium Detection

The substrate channel is formed by residues from the M and L subunits, which create a funnel leading to the active site. The active site contains the molybdenum ion with a distorted square pyramidal coordination environment. Transparent ribbons are colored as in Fig. 1 (left monomer). (B) Selenium identification by anomalous scattering detected at energies higher (λ = 0.9780 Å in red) and smaller (λ = 0.9803 Å in green) than the energy of the Se K edge. Both Bijvoet difference maps are contoured at the +5.0σ level. (C) F_DoC − F_Calc map for the Mo and pyranopterin cofactor at a contour level of +5.0σ. For the calculation of the map all atoms of the Mo ion and the pyranopterin cofactor were omitted.

Figure 2. The Mo-Se active site of NDH. (A) Stereoview of the active-site environment. Transparent ribbons are colored as in Fig. 1 (left monomer), (B) Selenium identification by anomalous scattering detected at energies higher (λ = 0.9780 Å in red) and smaller (λ = 0.9803 Å in green) than the energy of the Se K edge. Both Bijvoet difference maps are contoured at the +5.0σ level. (C) F_DoC − F_Calc map for the Mo and pyranopterin cofactor at a contour level of +5.0σ. For the calculation of the map all atoms of the Mo ion and the pyranopterin cofactor were omitted.

Structure-Based Reaction Mechanism of NDH. Increasing evidence on the chemistry of XORs supports a mechanism of substrate hydroxylation involving a base-assisted nucleophilic attack of the equatorial Mo–OH group on the substrate, with a concomitant hydride transfer from the substrate to the Mo(+IV)=Se group to give Mo(+IV)-SH. Two residues in the direct environment of molybdenum, Q208L in NDH (Q767 in XOR) and E289M (E1261 in bXOR), are conserved among the molybdenum hydroxylases. E1261 serves as general base catalyst in accepting the proton from Mo–OH upon reaction in bXOR (11) (Fig. S3) and, accordingly, replacement of this residue in bacterial XOR by alanine profoundly compromises the reactivity of the enzyme (35). Additionally, NDH and bXOR have common residues in the active site like R319L (R880 in bXOR) and F353L (F914 in bXOR) (Fig. S3). R880 of bXOR was suggested to stabilize the developing negative charge on the substrate during the hydroxylation step, and its mutation results in an increase of the dissociation constant, K_d, for substrate binding and a decrease in the rate constant of enzyme reduction, k_cat (36). The conservation of amino acids essential for catalysis indicates common ways of substrate binding and transition state stabilization in NDH and XORs. Based on these similarities (Fig. S3), we constructed a structural model for nicotinate binding in the active site of NDH in analogy to the structure of bXOR in complex with 2-hydroxy-6-methylpurine (27). It has recently been shown that 2-hydroxy-6-methylpurine binds between phenylalanine residues in the active site of bXOR, with the carbon atom to be hydroxylated in close proximity to the equatorial hydroxyl ligand. To allow binding of nicotinate in the active site of NDH the side chain of F353L has to rotate to assume a similar conformation as found for substrate/ligand-bound bXOR (21, 27) (Fig. S3). Modeling of substrate binding in analogy to bXOR produces a mechanistically reasonable complex. The nitrogen atom and carboxylate of nicotinate are at hydrogen-bonding distance to Y13_M and R319_M, respectively. C6, the carbon atom to be hydroxylated, is in a distance of ~2 Å from the hydroxyl ligand and 2.5 Å from the Se ligand. This binding mode would facilitate the nucleophilic attack of the hydroxyl ligand on C6 and the concomitant hydride transfer from C6 to the Se ligand (Fig. S4).

Computational Study Comparing Mo→Se and Mo→S. The functional advantage of selenium over sulfur as a ligand is not immediately understood.
The molybdenum sulfido ligand found in the active site of molybdenum hydroxylases like XOR (Mo\(\text{A}\)S) plays an important role in the catalytic activity of these enzymes. Its replacement by a (second) Mo\(\text{A}\)O group to give the so-called desulfo form of the enzyme leads to its complete inactivation.

The hydride transfer taking place in the initial step of the reaction can be considered a nucleophilic attack of the hydride on the Mo\(\text{A}\)E antibonding orbital (lower) and antibonding configurations, with the relative energies for Mo\(\text{A}\)O, Mo\(\text{A}\)S, and Mo\(\text{A}\)Se species indicated.

A Mo\(\text{A}\)Se rather than Mo\(\text{A}\)S is expected to yield an even weaker \(\pi\) bond with molybdenum than sulfur and oxygen (O > S > Se) (Fig. 3) and is thus expected to increase the reactivity of the enzyme in any mechanism involving hydride transfer. In the case of less reactive carbon centers, the increased reactivity may be important in catalyzing the hydroxylation.

The geometry of the optimized Mo\(\text{A}\)Se-containing structure is square pyramidal, with the Mo\(\text{A}\)E antibonding orbital (\(\pi^*\)) (E = O, S). The stronger interaction leads to higher \(\pi^*\) anti-bonding energy, which raises the barrier of the reaction. The strength of the Mo\(\text{A}\)O \(\pi\) bond relative to that of Mo\(\text{A}\)S leads to a very high \(\pi^*\) anti-bonding orbital and is presumably the basis for the lack of activity seen in the desulfo form of the enzymes (Fig. 3).

The calculations indicate that the transition state for the first step of the reaction is stabilized by 3.1–3.4 kcal/mol upon substitution of selenium for sulfur in the reaction with both ethylaldehyde (\(\Delta H^\ddagger\) is 3.47 kcal/mol for Mo\(\text{A}\)S and 0.13 kcal/mol for Mo\(\text{A}\)Se) and formamide (\(\Delta H^\ddagger\) is 14.81 kcal/mol for Mo\(\text{A}\)S and 11.67 kcal/mol for Mo\(\text{A}\)Se). This amounts to a factor of 200–300 in rate acceleration for this step.

The geometry of the optimized Mo\(\text{A}\)Se-containing structure is square pyramidal, with the Mo\(\text{A}\)E antibonding orbital (\(\pi^*\)) (E = O, S, Se). Depicted are the bonding interactions between the \(d_x\) orbital of the molybdenum and the \(p_x\) orbital of the coordinated chalcogen in bonding (lower) and antibonding configurations, with the relative energies for Mo\(\text{A}\)O, Mo\(\text{A}\)S, and Mo\(\text{A}\)Se species indicated.

### Discussion

The nature of the selenium moiety of NDH has been investigated over the last decade by using various approaches. After the discovery that selenium is essential for hydroxylase activity (13), Dilworth (14) showed that selenium is a component of NDH, which could be released by heat treatment or the addition of chaotropic agents like urea. That selenium in NDH is not part of a stable organic molecule but that is instead present as a neutral selenol or an inorganic selenide has been indicated by the liberation of selenium from NDH by incubation with alkylating agents, resulting in the formation of dialkylselenides (14). The approximate ratio of 1 mol of selenium per mol of NDH was established by EPR studies of NDH with the \(^{77}\text{Se}\) isotope that showed the nuclear spin of \(^{77}\text{Se}\) couples to the Mo(V) electron spin, suggesting that the selenium could be a ligand to molyb-
denum (18). This left the possibilities that selenium replaced the sulfido ligand at the molybdenum or that it could be weakly bound to a heteroatom of a separate cofactor adjacent to molybdenum. The crystal structure of NDH presented here shows that selenium indeed replaces sulfur as a molybdenum ligand, present as Mo=Se. The refined bond length of 2.3 Å agrees well with the expected bond length for such a terminal selenido ligand and specifically is too short for a selenol ligand (37). No further stabilization of the selenium by other interactions is observed, nor do we find residues such as cysteines in the vicinity of the Mo ion, which might form a bond to the selenido ligand. Access to the selenido ligand is partly blocked by F353L (Fig. 2A), which could explain why incubation of active NDH with potassium cyanide does not lead to a rapid inactivation of the enzyme and inactive enzyme could not be reactivated by the addition of sodium selenide or selenophosphate (18, 34).

Whether selenium is also a ligand to molybdenum in other selenium-dependent molybdenum hydroxylases remains to be established in future structural studies. For the selenium-containing XOR from E. barkeri it has been shown that the enzyme can be inactivated with potassium cyanide and enzyme thus inactivated can be reactivated by incubation with selenide under reducing conditions (17), observations that could be explained by the presence of a selenido ligand bound to molybdenum such as is seen here with NDH. However, with the purine hydroxylase from C. purinolyticum no magnetic interaction between the nuclear spin of 77Se and the Mo(V) electron spin could be detected, raising the possibility that the labile selenium is not coordinated to Mo in all forms of this enzyme (38). The catalytic advantage of the incorporation of selenium in a molybdenum hydroxylase is evident from comparing XORs of different organisms. While for bXORS with a sulfido ligand at the molybdenum turnover number of 2–25 s⁻¹ has been reported (39), the selenium containing XOR from E. barkeri achieves turnover rates >400 s⁻¹ (17). If the XOR from E. barkeri has a selenido ligand like NDH, the higher rates are likely caused by an acceleration of the hydride transfer step, as suggested by our model calculations.

For the molybdenum hydroxylase family we now see that at least 3 variations for the common theme MoO(OH)X coordination sphere exist, where X is: (i) S for XORs (40), quinoline oxidoreductase (24), and 4-hydroxybenzoyl-CoA reductase (23); (ii) SCu for carbon monoxide dehydrogenase (28); and, as detailed above (iii) Se for NDH. All active sites can be converted to an inactive state in which X is O, to give a second Mo=O ligand. These variations demonstrate how nature adapts a protein bound ligand-metal motif for different substrates and reactivities by the exchange of 1 ligand and indicate a flexible biochemical biology of molybdenum enzymes by ligand tuning.

Materials and Methods

**Purification of NDH.** As NDH is instable and loses activity with time (34) our experimental strategy was to take < 1 week from breaking the E. barkeri cells used to purify the protein until freezing the protein crystals used for structure determination. For all steps, including crystallization of NDH, buffer conditions were used for which NDH was reported to be most stable and active (34). Frozen E. barkeri cells were resuspended in 50 mM Tris-HCl (pH 7.8), 10 mM NaCl, 1 mM EDTA, and 2 mM DTT (buffer A) and broken by 5 cycles of sonication. After centrifugation for 30 min the cell-free extract was loaded on a Source 30Q column. NDH was eluted from the anionic exchange column by a linear gradient of buffer A containing 0.5 M NaCl. Pooled fractions were loaded onto a hydroxypapitate column without preceding buffer exchange. Active NDH eluted with 120 mM KPO4, pH 7.3. Active fractions were collected, rebuffered in 50 mM Tris-HCl (pH 7.8), 0.2 M KCl, 1 mM EDTA, and 2 mM DTT and concentrated to 20 mg/mL. The protein was stored at −4 °C and used within 5 days. Approximately 1.5 mg NDH could be obtained from 10 g of cells. To obtain protein with higher purity, an additional gelfiltration step (Superdex 200) was performed in 50 mM Tris-HCl (pH 7.8), 0.2 M KCl, 1 mM EDTA, and 2 mM DTT. All purification steps were carried out under anoxic conditions in a glove box containing 95% N2 and 5% H2.

**Activity Measurement.** Enzyme assays were conducted according to Gladyshev et al. (34) with some modifications. Hydroxylase activity was measured under anoxic conditions in 100 mM KPO4 (pH 7.0), 50 mM nicotinate (pH 7.3), 1 mM NADP⁺, and 5 mM DTT. The reaction was started by addition of NADP⁺ and followed by absorption increase at 340 nm.

**Crystallographic Methods.** NDH was crystallized by the vapor diffusion method in a hanging drop. The enzyme preparations used for crystallization had specific activities of 11–20 units mg⁻¹. The drop contained a 1:1 mixture of protein solution (10 mg/mL) with reservoir solution containing 18–20% PEG 3350, 0.1 M Tris-HCl (pH 7.5), 75 mM NaNO3, 5% glycerol, or 1% 2,4-methylpentanitril. Crystals were harvested in the corresponding soaking liquid contained 15% (vol/vol) (2,3)-butanediol (Sigma), shock-frozen, and stored in liquid nitrogen. Diffraction data were collected at −180 °C on a rotating anode X-ray generator (Nonius FR591; Brüker) equipped with an image plate detector (mar345/dtb; Mareresearch) (Table 1) and at the BM-14, European Synchrotron Radiation Facility, Grenoble (above and below 5e-edge, Table 1). The protein structure model was build with Coot (41) and MAIN (42). Positional and temperature refinement was carried out with CNS (43). Anomalous different Fourier maps were calculated by using CNS (43). The final refinement statistics and stereochemical analyses using PROCHECK (44) are shown in Table 1. Ramachandran statistics revealed 86% in the most favored, 13% in the additionally allowed, 1% in the generously allowed, and 0% in the disallowed regions. Simulated annealing omit maps were used to validate the active-site structure reported.

**Model Calculations.** Calculations were carried out by using hybrid density functional theory (B3LYP) as implemented in Gaussian 03. The structures were fully optimized and confirmed as minima or transition state by calculating the vibrational frequency. The structures were optimized with B3LYP using LANL2DZ ECP (45, 46) augmented with fpolarization functions (47) for Mo and 6–31G(d) for the rest of the atoms. For the hydrogen that performs the hydride transfer 6–31G(d,p) was used. The zero point energies were calculated with same basis set and level of theory. All quoted energies are corrected to zero point energy and are without temperature correction.

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Supporting Information

Wagener et al. 10.1073/pnas.0902210106

SI Text

Searches in finished and unfinished genomic data yielded 3 bacterial species with gene clusters in which all 9 enzymes of the nicotinate fermentation pathway of *E. barkeri* (1, 2) could be identified. In *Natranaeobius thermophilus* JW/NM-WN-LF (3) the Mo-pyranopterin binding subunit is also split into 2 polypeptides. *Anaerotruncus colihominis* DSM 17241 (4) and *Bacteroides capillosus* ATCC 29799 (5) have fused Mo-pyranopterin binding subunits. The alignment (Fig. S1) shows the amino acid sequence identity among split, fused and between split and fused subunits. In full agreement with the genetic context amino acid identities within this group are high (50–86%, with an average of 61%).

Excluding obvious sequencing errors and incomplete contigs, 11 split Mo-pyranopterin binding subunits were found in finished and unfinished genomes. Sequencing errors are unlikely to have caused the splitting in these cases. In various organisms and proteins the splitting occurs at a similar position and clear-cut Shine-Dalgarno sequences are present at appropriate distances from the startcodon of the NdhM homologs (see Fig. S2). These subunits are unlikely to belong to NDHs as their amino acid sequence identity with NDHs (30–47%, with an average of 36%) is lower than that among the NDHs mentioned above. Annotations in the databases usually list "carbon monoxide dehydrogenase CoxL/CutL homolog," but comparison with authentic sequences of CoxL from *Oligotropha carboxidovorans* OM5 and CutL from *Hydrogenophaga pseudoalga* shows only 23–28% amino acid sequence identity. For the *Carboxydothromus hydrogenoformans* case the assignment to an aerobic carbon monoxide dehydrogenase was already dismissed (6). Amino acid sequence identities with structurally and biochemically well-characterized Mo-pyranopterin containing proteins are low and do not reveal a possible role for these split subunit enzymes: 24−34% with 4-hydroxybenzoyl-CoA reductase from *Thauera aromatica*, 26−32% with *Desulfovibrio desulfuricans* or *Desulfovibrio gigas* aldehyde oxidoreductases, 22–29% with bovine or *Rhodobacter capsulatus* xanthine dehydrogenases, and 25–30% with quinoline 2-oxidoreductase from *Pseudomonas putida*. We suspect that the split Mo-pyranopterin binding subunits belong to hydroxylases of aromatic substrates such as purine.

In summary, the combined findings point out that split Mo-pyranopterin binding subunits are not a singularity in *E. barkeri* NDH.

Fig. S1. Evidence for split and fused Mo-pyranopterin binding subunits of NDH. ClustalW amino acid sequence alignment of the subunits present in gene clusters harboring all 9 enzymes of the nicotinate fermentation pathway (1). The intensities of the blue highlighting shades are proportional to the amino acid sequence identities (50%, 75%, 100%; no highlighting, all differ).

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Fig. S2. Nucleotide sequences and translations of the region corresponding to the C terminus of NdhL and N terminus of NdhM in a genetic context pointing toward purine degradation. C termini of NdhL (-homologous) subunits are highlighted in red; N termini of NdhM highlighted in black, and start codons are highlighted in green.

```
30 f7
R  R  P  C  D  H  I  K  V  Q  G  R  D  N  K
E  E  A  L  R  S  Y  Q  G  P  G  E  G  Q  *
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G  S  C  S  Q  V  S  K  Y  L  G  G  G  R  V
M  E  A  V  A  K  S  L  N  I  L  G  E  V  E  *
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R  A  N  A  L  F  V  A  L  R  F  Q  E  A  L
S  G  P  M  R  F  S  S  L  S  G  F  R  R  R  *
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Cggcgcaacgcggttctcatcggtgttcggcacgaggagg
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G  A  T  R  F  S  S  V  F  G  T  R  R  R  *
cggcgccacccggttctcatccgtgttcggcacgaggagg
R  R  N  I  W  R  C  P  P  T  G  R  E  P  D
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T  G  S  F  K  A  F  K  R  E  R  R  C  S  T
I  Q  E  A  L  K  L  S  K  E  K  E  G  V  R  H  *
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H  F  G  W  N  V  E  E  L  K  *
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cgagataaagccgaagaaatttttggagatgaagggagg
K  H  W  K  K  P  E  K  K  Q  W  R  *  W  V
 cgaaacactggaaaaagccagagaaaaagcagtggagg
Eubacterium barkeri
AG RK P A A S P I R C R N R H G G G D A S E Q F L A L C R
AG Q C A F R R S Q V S G G A D E E P W V E R R R R W N L S F T V P

37 (Sse37_21550 and Sse37_21545, Genbank acc. AAYA00000000)

SJ95 (Pmob_1740 and Pmob_1741, Genbank acc. CP000879, TTG as startcodon for Pmob_1741)

ORS278 (Brado4775 and Brado4774, Genbank acc. CU234118)

MAFF303099 (Mlr1703 and Mlr1704, Genbank acc. BA000012)

DSM 1223 and

DSM 15981 (CLOSTASPAR_04924 and CLOSTASPAR_04923, GenBank acc. ACCJ01000408)

MAFF303099 (Mlr1703 and Mlr1704, Genbank acc. BA000012)

USDA 110 (Bll3375 and Bll3374, Genbank acc. BA000040)

QYMF (Amet_4569 and Amet_4568, Genbank acc. CP000724)

JW/NM-WN-LF (Nther_2151 and Nther_2150, Genbank acc. CP001034)

OhILAs (Clos_0377 and Clos_0378, Genbank acc. CP000853)

ATCC 39073 (Moth_1226 and Moth1225, Genbank acc. CP000232)

DSM 15981 (CLOSTASPAR_04924 and CLOSTASPAR_04923, GenBank acc. ACCJ01000408)

Bradyrhizobium japonicum

Bradyrhizobium sp.

Bradyrhizobium sp. sp. ORS278 (Brado4775 and Brado4774, Genbank acc. CU234118)

Bradyrhizobium sp. sp. BTa1 (Bhta_4809 and Bhta_4808, Genbank acc. CP000494)

Bradyrhizobium japonicum USDA 110 (Biis375 and Biis374, Genbank acc. BA000040)

Sagittula stellata E-37 (Sese21_2150 and Sese37_21545, Genbank acc. AAYA00000000)

Alkaliphilus oremlandii OhLAs (Clo3_0377 and Clo3_0378, Genbank acc. CP000853)

Alkaliphilus metalireducens QYMF (Amet_4569 and Amet_4568, Genbank acc. CP000724)

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Fig. S3. Comparison of the active sites of NDH (red) and xanthine oxidoreductase (blue) (Protein Data Bank ID code 1FO4) (8). The salicylate molecule bound in the active site has been marked as Sal.
Fig. S4. Model for nicotinate binding in the active site. The model was constructed in analogy to the substrate and inhibitor structures of xanthine oxidoreductase (8–11). To allow for substrate binding the conformation of F353 was changed to the conformation found for F914 in xanthine oxidoreductase (Fig. S3) (8).
Fig. S5. Geometry of the optimized molybdenum active site model of xanthine oxidoreductase (Left) and its selenium analogue (Right).
Table S1. Selected bond length (Å) and bond angles (°) of LMo(OH)E (E = S,Se) (L = 2-butene-2,3-dithiolate) as molybdenum active-site model

<table>
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<th>Bond</th>
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Experimentally determined values were taken from Doonan et al. (7).