Nickel-based Enzyme Systems

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Of the eight known nickel enzymes, all but glyoxylase I catalyze the use and/or production of gases central to the global carbon, nitrogen, and oxygen cycles. Nickel appears to have been selected for its plasticity in coordination and redox chemistry and is able to cycle through three redox states (1+, 2+, 3+) and to catalyze reactions spanning \( \sim 1.5 \) V. This minireview focuses on the catalytic mechanisms of nickel enzymes, with an emphasis on the role(s) of the metal center. The metal centers vary from mononuclear to complex metal clusters and catalyze simple hydrolytic to multistep redox reactions.

Seven of the eight known nickel enzymes (Table 1) involve the use and/or production of gases (CO, CO\(_2\), methane, H\(_2\), ammonia, and O\(_2\)) that play important roles in the global biological carbon, nitrogen, and oxygen cycles (1). CODH\(^2\) interconverts CO and CO\(_2\); ACS utilizes CO; the nickel ARD produces CO; hydrogenase generates/utilizes hydrogen gas; MCR generates methane; urease produces ammonia; and SOD generates O\(_2\).

The nickel sites in enzymes exhibit extreme plasticity in nickel coordination and redox chemistry. The metal center in SOD must be able to redox processes with potentials that span from +890 to \(-160 \) mV (2), whereas in MCR and CODH, it must be able to reach potentials as low as \(-600 \) mV (3); thus, nickel centers in proteins perform redox chemistry over a potential range of \( \sim 1.5 \) V.

Because natural environments contain only trace amounts of soluble Ni\(^{2+}\), attaining sufficiently high intracellular nickel concentrations to meet the demand of the nickel enzymes requires a high affinity nickel uptake system(s) (4), molecular and metallochaperones (5), and sensors and regulators of the levels of enzymes involved in nickel homeostasis (6). However, space limitations prevent coverage of these pre-catalytic events.

Glyoxylase I

GlxI and GlxII catalyze conversion of methylglyoxal, a toxic species that forms covalent adducts with DNA, to lactate (Table 1, Reaction 1) (7). A single nickel ion in an octahedral coordination environment acts as a Lewis acid catalyst and remains in the 2+ state throughout the isomerization reaction (supplemental Fig. S1), which is consistent with the utilization of zinc at the GlxI active site in some organisms, including humans (8). The Ni\(^{2+}\) ion binds the hemithioacetal adduct between methylglyoxal and GSH, displacing a water ligand. General base catalysis by Glu\(^{122}\) leads to proton abstraction from the substrate, forming the coordinated enediol intermediate. Reprotonation at C-2 promotes generation of the product S-D-lactoylglutathione, which undergoes hydrolysis to lactate and GSH in a separate reaction catalyzed by GlxII.

Acireductone Dioxygenase

ARD performs the penultimate step in the methionine salvage pathway (Table 1, Reaction 2) (9, 10). ARD belongs to the cupin superfamily, and the structure reveals an octahedral high spin Ni(II) center, hexacoordinated by three histidines, one aspartic acid, and two waters (supplemental Fig. S2). Nickel neither undergoes redox changes nor binds O\(_2\); instead, the substrate acireductone (A, 1,2-dihydroxy-3-oxo-5-(methylthio)pent-1-ene) reacts directly with O\(_2\) to form the peroxo species (B), and nickel remains in the 2+ state throughout the reaction, like Cu\(^{2+}\) in the mechanism of copper amine oxidases (11). Nickel acts as a Lewis acid, promoting attack by the peroxo intermediate on the nickel-ligated carbonyl group to generate a cyclic intermediate (D) that decomposes to CO, formic acid, and a carboxylic acid.

Nickel Superoxide Dismutase

SOD emerged with the rise in O\(_2\) levels around 2 billion years ago (12) as part of a cellular defense system against reactive oxygen species generated by various reactions associated with oxygen metabolism, including respiration and oxidative stress events associated with macrophage and neutrophil infection (13, 14). SOD targets superoxide (Table 1, Reaction 3), which directly destroys iron-sulfur clusters in redox enzymes, and reacts with nitric oxide to generate peroxynitrite, a powerful oxidant and nitrating agent (15).

There are multiple SODs, the Cu/Zn-SODs, the Fe-SODs, the Mn-SODs, the cambialistic SODs (which can function with either manganese or iron), and the Ni-SODs, all of which catalyze the conversion of superoxide to O\(_2\) and H\(_2\)O\(_2\) (Table 1, Reaction 3), with catalytic efficiencies \( k_{cat}/K_m \sim 10^9 \) M\(^{-1}\) s\(^{-1}\) near the diffusion limit (14, 16). Originally isolated from Streptomyces coelicolor (17), the Ni-SOD gene (sodN) has been found in cyanobacteria, marine gammaproteobacteria, and a marine eukaryote (18).

Ni-SOD switches between a square planar \( \text{Ni}_2\text{S}_2\) (Ni\(^{2+}\)) and square pyramidal \( \text{Ni}_2\text{S}_3\) (Ni\(^{3+}\)) (supplemental Fig. S3) coordination environment (19), composed of the sulfur atoms of two Cys residues and two peptide backbone nitrogens. Proteolytic processing of an inactive proprotein seeds the formation of the so-called nickel hook, a conserved 12-amino acid sequence that provides all of the essential interactions for metal binding (19), and a catalytically active six–residue maquette has been synthesized (20). The Cys sulfur ligands appear to poise the Ni\(^{2+}\)/2+ redox couple in the appropriate range for catalyzing both the
reduction and oxidation of superoxide and to serve as a proton donor during catalysis (19, 21).

The SOD reaction involves the binding of superoxide to the Ni\(^{2+}\) center, generating a Ni\(^{3+}\)-peroxo species that undergoes proton and electron transfer to generate \(\text{H}_2\text{O}_2\) and the oxidized Ni\(^{3+}\) center. Binding of a second superoxide generates a Ni\(^{3+}\)-peroxo intermediate, which donates an electron back to the Ni\(^{3+}\) center to liberate dioxygen and re-form the starting Ni\(^{2+}\) state.

**Urease**

Urease is key to the global nitrogen cycle because it catalyzes hydrolysis of urea, which is excreted by vertebrates, into ammonia and bicarbonate (Table 1, Reaction 4) (22, 23). Thus, urease is absent in vertebrates but facilitates nitrogen assimilation by plants, algae, and bacteria, a role that is underscored because urea is a major globally used soil fertilizer. Urease is also a virulence factor for pathogens in the animal gut and urinary tract, promoting host colonization by neutralizing the low pH in the stomach (24).

Many structures of urease and site-directed variants in the presence and absence of substrates and inhibitors are available (22). Urease contains a dinickel center, with Ni1 encased in a square pyramidal N\(_2\)O\(_3\) environment and Ni2 in a pseudo-octahedral N\(_2\)O\(_4\) arrangement (supplemental Fig. S4). Ni1 is bridged to Ni2 through a water/hydroxyl group (\(\text{Wb}^\text{18572}\)) and a carboxylate moiety from \(\text{N}\)-carboxyllysine (22). Remaining in the 2+ state, the bimetallic center acts as a Lewis acid role, providing a 10\(^{14}\)-fold rate enhancement of urea hydrolysis, with \(k_{\text{cat}}\) values as high as 3000 \(s^{-1}\) and \(k_{\text{cat}}/K_m\) of 10\(^6\) \(M^{-1} s^{-1}\). In the enzymatic mechanism, urea binds with its carbonyl oxygen bound to Ni1; then, when a flap in the protein closes, urea bridges the two nickel ions, with one of the amino groups bound to Ni2 and the distal amino group interacting with H-bond acceptors (His\(^{320}\), Ala\(^{363}\), and Gly\(^{277}\)) in the active site. Transfer of a proton to the “free” distal amino group promotes attack of water on the urea carbonyl group, leading to formation of ammonia and carbamate, which spontaneously hydrolyzes into bicarbonate and another molecule of ammonia. As two water molecules bind, ammonia and carbamate dissociate to re-form the catalytic center.

**NiFe Hydrogenase**

Hydrogenases catalyze the reversible two-electron reduction of protons to \(\text{H}_2\) (Table 1, Reaction 5) (25, 26). Anaerobic microbes remove \(\text{H}_2\) from the environment and couple its oxidation to the reduction of various terminal electron acceptors (e.g. \(\text{O}_2\), \(\text{NO}_3^-\), \(\text{SO}_4^{2-}\), \(\text{CO}_2\), and fumarate) (26). Microbes also contain \(\text{H}_2\)-evolving hydrogenases, siphoning off excess cellular reducing equivalents by reducing protons to \(\text{H}_2\).

Besides the NiFe hydrogenase, there are FeFe (primarily involved in \(\text{H}_2\) evolution) and iron hydrogenases (26). Of the four classes of NiFe hydrogenases, one is a membrane-associated proton-pumping and energy-coupling complex (26). All NiFe hydrogenases contain at least two subunits (“large” and “small”), with the 60-kDa large subunit containing the binuclear NiFe active site (Fig. 1) that is coupled to a “wire” within the 30-kDa small subunit, which contains one to three Fe-S
clusters (25). The iron subsite of the NiFe center contains one CO and two cyanide ligands, which are thought to maintain iron in its low spin ferrous state. There also exists a H₂-sensing protein that shares the NiFe active site, including the CN/CO ligands, but has minimal catalytic activity and interfaces with a two-component signal transduction system to control the expression of hydrogenase-related genes (27).

Several mechanisms have been proposed to explain the hydrogenase-catalyzed reaction (26, 28). H₂ oxidation is diffusion-controlled (k_cat/K_m ≈ 10⁸–10⁹ M⁻¹ s⁻¹), with a turnover number reaching 9000 s⁻¹ at 30 °C. Because the NiFe catalytic center is buried 30 Å beneath the surface of the protein, H₂ must travel through a tunnel in the protein to reach and react with the binuclear active site (29).

The NiFe hydrogenase requires activation, involving prolonged treatment with H₂ to generate the Ni₄-C⁺ state, perhaps involving replacement of an OH⁻ ligand with a hydride bridge (in red) between the nickel and iron sites (Fig. 1) (25). Activation appears to involve heterolytic H⁻H bond cleavage (28). Catalysis ensues upon conversion of the Ni₄-C⁺ state to a Ni(I) oxidation state (Ni₄-R⁺) by a hydride transfer or proton-coupled electron transfer reaction, allowing productive binding of H₂ (28). H⁻H bond cleavage during the catalytic cycle is proposed to occur by an oxidative addition mechanism that would generate the Ni₄-X⁺ intermediate, which undergoes two successive proton-coupled electron transfer steps to regenerate Ni₄-C⁺ (28).

CO Dehydrogenase

CODH catalyzes the reversible oxidation of CO to CO₂ (Table 1, Reaction 7), allowing anaerobic microbes to grow with CO or CO₂ as their sole carbon source and with CO₂ as the only energy supply (30, 31). The rate of CO oxidation to CO₂ varies widely among CODHs, with the nickel-containing Carboxydothermus hydrogenoformans enzymes holding the current speed record of 40,000 s⁻¹ and diffusion-controlled k_cat/K_m of 2×10⁹ M⁻¹ s⁻¹ (65 °C). Remarkably, at low pH values, CO₂ reduction can exceed the rate of CO oxidation (32). Microbes remove and oxidize 10⁸ tons of CO from earth's lower atmosphere every year, helping to maintain low ambient CO levels. Apparently, microbes can even cycle CO as an intermediate in bioenergetic cycles and couple CO oxidation to H₂ production. CO is likely to have been relatively abundant in the early earth, and the CODH and ACS reactions are speculated to have been key to the evolution of life (33).

There exist a monofunctional nickel CODH, containing 10 irons and 1 nickel per monomer, and a bifunctional CODH/ACS, containing 14 irons and 3 nickels (30, 31). The nickel CODH is a mushroom-shaped homodimer containing five metal clusters (two B- and two C-clusters and one D-cluster). The catalytic C-cluster (supplemental Fig. S5) is a [3Fe-4S] cluster bridged to a binuclear NiFe cluster and is buried 18 Å below the protein surface. The B- and D-clusters are [4Fe-4S]¹⁺/¹⁻ clusters that act as a wire to transfer electrons between the C-cluster and external redox proteins, like ferredoxin.

Step 1 of the proposed mechanism (34) involves CO binding to nickel and water to iron in the binuclear component of the C-cluster, followed by deprotonation of the bound water to generate an active hydroxide. In Step 2, the OH group attacks the metal-CO complex to form a nickel-carboxylate complex that was shown by x-ray crystallography to bridge the nickel and iron atoms (35). In Step 3, which ends the ping phase of this ping-pong reaction, CO₂ is generated and released, as two electrons are internally transferred to the C-cluster. Step 4 involves electron transfer from the two-electron reduced C-cluster to the B- and D-clusters in the redox chain; and finally, in the pong step, electrons are transferred from CODH to ferredoxin or other electron carrier proteins.

Acetyl-CoA Synthase

The active site of ACS is the A-cluster, a [4Fe-4S] cluster that is thiolate-bridged (by Cys509) to the proximal nickel (Ni₄₋), which in turn is thiolate-bridged to Ni₄₋ (supplemental Fig. S6). The coordination environment of Ni₄₋ resembles the active site of Ni-SOD, and the overall arrangement is similar to that of the iron-only hydrogenases in which a [4Fe-4S] cluster is bridged to a diiron site (37).

Details of the ACS reaction have been reviewed (1, 30, 31), and two general mechanisms of acetyl-CoA synthesis have been proposed, which differ in the assignment of the redox states of Ni₄₋ during catalysis and the order in which the CO and CH₃ groups bind to the enzyme. However, recent studies indicate that CO and CH₃ bind randomly (38). Regardless, both proposals invoke three organometallic intermediates (supplemental Fig. S6) on Ni₄₋ with Ni₄₋ remaining a Ni(II) bystander throughout the catalytic cycle. Crystallographic studies demonstrate that CODH/ACS can assume a closed (39) and an open (40) state that likely alternate during catalysis.

With CO as the first substrate, ACS apparently initiates catalysis from the “closed” state as CO moves through the channel and binds to Ni₄₋, forming an organometallic nickel-CO complex that has been well characterized (31). Then, as ACS assumes the “open” conformation, the methyl group from the methylated CFeSP is transferred to the A-cluster, followed by condensation of the methyl and carbonyl groups to form an acetyl-metal species. Finally, CoA binds, triggering thiolysis of the acetyl–metal bond to form acetyl-CoA.

Methyl-CoM Reductase

All biologically generated methane on earth derives from the catalytic activity of MCR in methanogenic microbes. An MCR isozyme also appears to catalyze anaerobic methane oxidation (41). MCR catalyzes the conversion of methyl-CoM (methyl-CoM) and N₇-mercaptoheptanoylthreonine phosphate (CoBSH) to methane and the CoB-SS-CoM heterodisulfide (Table 1, Reaction 6) (42, 43). Methane formation by MCR
occurs with a turnover number of ~100 s⁻¹ and a $k_{cat}/K_{m}$ (methyl-SCoM) of ~$1 \times 10^5$ M⁻¹ s⁻¹ (44, 45).

Based on crystal structures of the inactive Ni(II) enzyme, MCR consists of an ($\alpha\beta\gamma$) structure, with its catalytic center, a nickel hydrocorphin called coenzyme $F_{430}$ in the $\alpha$-subunit (Fig. 2) (46). Containing only five double bonds, $F_{430}$ is the most reduced tetrapyrrole in nature, and only the Ni(I) form of the enzyme can initiate catalysis.

Two general catalytic mechanisms are under discussion, and the experimental basis for each mechanism has been reviewed (42, 43). Mechanism I involves an organometallic methyl-nickel intermediate, and Mechanism II invokes a methyl radical (Fig. 2).

In Step 1 of Mechanism I, Ni(I) performs a nucleophilic attack on methyl-CoM to form a methyl-Ni(III) intermediate and to release CoM. In Step 2, electron transfer from CoM to the alkyl-Ni(III) generates alkyl-Ni(II) and a CoM radical. Then, in Step 3, the CoM radical reacts with CoBSH to form a disulfide radical anion as proton transfer from CoM to the bound methyl group generates Ni(II) and methane. Finally, in Step 4, the radical anion reduces Ni(II), yielding the CoM-SS-CoB heterodisulfide and active Ni(I) enzyme.

According to Mechanism II, Ni(I) reacts with methyl-SCoM at sulfur, which promotes cleavage of the C–S bond, generating a methyl radical, with the sulfur of CoM forming a Ni(II)-thiol adduct. In Step 2, the methyl radical abstracts a hydrogen atom from CoBSH to form methane and a thyl radical on CoB. Step 3 involves formation of Ni(II) and the disulfide anion radical, and Step 4, as above, regenerates the active Ni(I) state and the heterodisulfide. In Mechanism II, the major role of nickel is to facilitate C–S bond cleavage by a redox process and to stabilize the product of C–S homolytic bond cleavage by forming a coordination complex with the sulfur of CoM.

Perspective

Much has been learned about nickel-based catalysis since the discovery of the first nickel enzyme (47) mainly because the nickel center is amenable to many biochemical and biophysical methods, allowing kinetic and spectroscopic characterization of catalytic intermediates in some of the eight enzymes described above. Further work will resolve some mechanistic controversies, elucidate the players in nickel trafficking within the cell, and uncover new nickel enzymes. Although studies of nickel depletion in rats indicated that nickel is an essential element for higher animals (48) and although nickel is present at ~0.5 nM in the human bloodstream (49), neither the source of the nickel requirement nor a single mammalian nickel-dependent enzyme has been identified.

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