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Effect of the Methyl-Coenzyme-M Reductase Protein Matrix on the Hole-Size and Nonplanar Deformations of Coenzyme F430

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Methyl-coenzyme-M reductase (MCR) is a key enzyme common to all methane-producing pathogens. It catalyses the final step in methane synthesis. Each MCR contains two noncovalently bound molecules of cofactor F430. Normal-coordinate structural decomposition, hole-size analysis, and molecular mechanics calculations were undertaken to examine the effect of MCR on the hole-size and nonplanar deformations of coenzyme F430. In MCR, the protein prevents F430 from undergoing nonplanar deformations, which results in a more rigid tetrahydrocorphinoid cofactor that has a shorter ideal metal–nitrogen distance in the MCR protein matrix than it does in solution. Changing the coordination number of the nickel ion in F430 has a very small effect on the ideal hole size; however, it has a significant effect on the nonplanar deformations the coenzyme undergoes upon contraction and expansion. In all complexes we examined, cofactor F430 undergoes more nonplanar deformations when it contains a four-coordinate metal ion than it does when it contains a six-coordinate metal ion. Clearly, MCR moderates the hole-size and the nonplanar deformations of coenzyme F430, which are known to affect redox potentials and axial ligand affinities. This suggests that the protein environment may be responsible for tuning the chemistry of the active-site nickel ion.

Introduction

The role of the protein in regulating the catalytic properties of metalloenzymes is the subject of great interest and debate.[1−4] The induced-fit and entatic state theories have been used to describe how the protein matrix surrounding a metal ion regulates the function of a metalloenzyme by exerting strain on the active-site metal and changing its electronic and steric properties. In this paper, we describe how the protein methyl-coenzyme-M reductase (MCR) modulates the nonplanar deformations of cofactor F430, thereby significantly changing the hole size and flexibility of the corphinoid coenzyme, which affects the chemistry of the active-site nickel ion.

Methyl-Coenzyme-M Reductase. Anaerobic bacteria produce 400 million tons of methane annually. About 45 million metric tons of the methane escape into the troposphere and significantly contribute to the greenhouse effect.[5] MCR is a key enzyme common to all methane-producing pathogens. It catalyzes the last step of methanogenesis in which methyl-coenzyme M (Me-CoM) and coenzyme B are joined by the formation of a disulfide bond (CoM−S−S−CoB) and methane is released. Recently, indirect evidence that MCR also catalyzes the anaerobic oxidation of methane has been reported.[6]

Crystal structures of MCR from *Methanobacterium marburgensis*, *Methanosarcina barkeri*, and *Methanopyrus kandleri* have been published.[7−9] Each MCR is composed of three subunits in a $(\alpha\beta\gamma)_2$ structure and contains two noncovalently bound molecules of cofactor F430 that are located about 50 Å from each other. This unusual nickel tetrahydrocorphinoid cofactor, Figure 1, is most likely the active site of MCR.[10] It has been suggested that the binding

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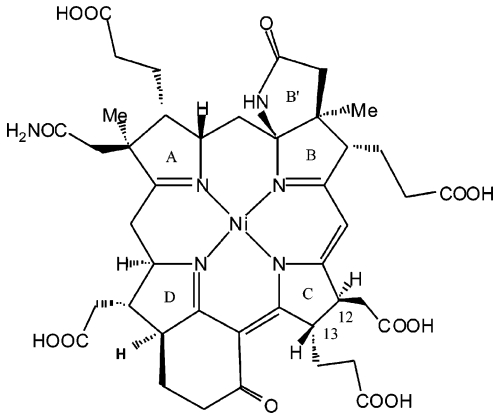

Figure 1. Structure of cofactor F430. Diepimerization occurs at positions 12 and 13.

of Me-CoM and coenzyme B to one active site induces a conformational change that is required to expel the product heterodisulfide from the second site into the water phase (somewhat like a two-stroke engine).[11] Two catalytic mechanisms for methane generation have been proposed.[7,10,12−14]

The cofactor F430 is held in place within the protein via hydrogen bonding to the partially negatively charged carboxylate groups of the Ni-porphrinoid.[15] The nickel ion is redox active and is found in nickel(I) EPR visible and nickel-(II) EPR silent forms.[16−20]

Free cofactor F430 is thermally unstable, it first epimerizes to 13-epi F430 and then in a second epimerization to 12,13-diepi F430.[21] Because of problems in the purification procedure, the crystal structure of free F430 has not been solved; however, the structure of a methanolated derivative, 12,13-diepi F430M, has been published.[22] Free, reduced F430M does not react with Me-CoM,[23] which leads us to assume that either F430 or Me-CoM are activated by MCR. Resonance Raman studies have shown that cofactor F430 undergoes a significant conformational change when it binds MCR.[20]

Nonplanar Deformations. At one time, it was thought that the aromatic porphyrin macrocycle would be planar. In fact, early structure determinations constrained the macrocycle to be planar. However, when high-quality crystallographic determinations of porphyrins and metalloporphyrins began to appear, it was soon obvious that the porphyrin ring was subject to a number of distortions and was often distinctly nonplanar. This nonplanarity of porphyrins is biologically relevant[24] and influences the chemical properties of porphyrin complexes.

Most deformations of the porphyrin core are in a direction perpendicular to the tetraaza plane. They can be classified into six classes. These are based on simple symmetric deformations, one of each out-of-plane symmetry classification of the (D_{4h}) point group of the square-planar macrocycle. More complicated asymmetric distortions that are composed of combinations of these simple distortions are also found.

A normal-coordinate structural decomposition (NSD) procedure has been derived to characterize and quantify porphyrin deformations in proteins.[24,25] The method determines the out-of-plane distortions in terms of the equivalent distortions along the lowest-frequency normal coordinates of the porphyrin.[26]

Nonplanar deformations are important because numerous studies have shown that nonplanar distortions have a significant effect on the chemistries of tetrapyrrole complexes.[25] It has been suggested that the nonplanar deformations observed in photosynthetic proteins are responsible for the photophysical and redox properties of chlorophyll pigments.[27] Nonplanar porphyrins are easier to oxidize than planar porphyrins.[27−29] Excited-state lifetimes of porphyrins are influenced by nonplanar deformations,[28,30] as is the axial ligand affinity.[31]

Resonance Raman studies have shown that in solution nickel protoporphyrin is found in an equilibrium mixture of nonplanar and planar conformations. However, when the nickel protoporphyrin is bound to hemoglobin, it is only found in the planar conformation, even when the nickel is not coordinated to the proximal histidine.[32]

We have previously shown that MCR modifies the reactivity of the nickel active site by changing the conformations available to cofactor F430 in two ways: by preventing cofactor F430 from adopting the energetically more favorable 12,13-diepimeric conformer and by moderating the nonplanar deformations F430 can adopt.[33]

In this paper, we will describe our work to determine the effect the protein, MCR, has on the hole size of cofactor F430. Since low-spin Ni(II)−$N_{pyrrole}$ distances are typically

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between 1.85 and 1.91 Å, high-spin six-coordinate Ni(II)−$N_{pyrrole}$ distances are between 1.98, and 2.05 Å and six-coordinate Ni(I)−$N_{pyrrole}$ distances are even longer with Ni(I)−$N_{pyrrole}$ distances of about 2.1 Å, the hole size will influence the redox potential. This is particularly important since MCR is a redox-active protein in which the inactive nickel(II) form is reduced to the active nickel(I) species.

Experimental Section

Molecular Mechanics. The AMBER* option of MacroModel v9.0016[34] was used with the default equations in all the molecular mechanical calculations. The parameters used to model the inorganic interactions were based on those we used in our previous calculations[33,35,36] and that were originally reported for complexes of low-spin and high-spin Ni(II) with polyamine ligands by Hancock.[37,38] Convergence was considered complete when the gradient obtained was <0.001 kJ/mol. The crystal structures of the MCR were downloaded from the Protein Data Bank.[39] Unless stated otherwise, the $MCR_{ox1-silent}$ state (pdb code = 1MRO) was used as the starting point for calculations. A hot-sphere of 8.00 Å around coenzyme F430 was used, all atoms in this substructure were minimized without restriction. Two subsequent shells extending a further 2.00 Å each were constrained to their *x*, *y*, and *z* coordinates by a force constant of 200.00 kcal/mol·Å^2 for the first shell and a force constant of 400.00 kcal/mol·Å^2 for the second shell. For the six-coordinate F430 structure in MCR, the nickel ion was bound to Gln147 and coenzyme M(2-mercaptoethanesulfonic acid), while in the absence of the protein matrix, a six-coordinate nickel ion was generated by coordinating two methyl mercaptan groups to the nickel ion.

Hole-Size Calculations. To determine the ideal average nickel−nitrogen distance of the F430 cavity, the molecular mechanics method of Hancock was used.[40−42] The ideal nickel−nitrogen distance in the AMBER* force field of MacroModel v9.0016 was systematically varied by 0.02 Å increments before energy minimization with 1000 iterations and no solvent. Ni−N force constants of 287 and 2000 kcal/mol·Å were both used (the size of the force constant made no difference). Convergence was considered complete when the gradient obtained was <0.001 kJ/mol. Hole-size calculations of F430 in MCR were carried out with an 8 + 2 + 2 Å hot-sphere as described in the molecular mechanics section. The strain energy of F430 in MCR, plotted in graphs such as those shown in Figure 2, was determined by calculating the single-point strain energy of F430 obtained from the minimized "F430 in MCR" structures. All structures were subjected to a conformational search before being subjected to a hole-size scan.

Conformational Searching. Conformational searches were conducted using the Monte Carlo (MC) torsional and molecular position variation method.[43,44] F430 was randomly rotated by between 0° and 180° and randomly translated by between 0 and 1.00 Å in each MC step.[45] The flexible dihedral angles of the side-chains of residues 120, 147, 153, 230, 330, 331, 333, 367, 396, and 443 were also randomly rotated by between 0° and 180° in each MC step. Fifteen thousand MC steps were taken in each search. Structures within 50 kJ/mol of the lowest-energy minimum were kept, and a usage directed method[44] was used to select structures for subsequent MC steps. Structures found in the conformational search were considered unique if the least-squaresd superimposition of equivalent non-hydrogen atoms found one or more pairs separated by 0.25 Å or more.

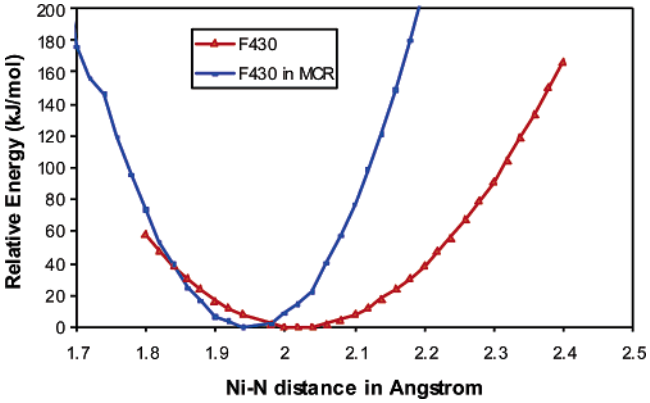

Figure 2. Relative strain energy (kJ/mol) for a six-coordinate metal ion bound to F430 (▲), and for a six-coordinate metal ion bound to F430 in MCR (■), as a function of the ideal M−N bond length calculated as described by Hancock.[40]

Normal-Coordinate Structural Decomposition. The nonplanar deformations of cofactor F430 were analyzed by NSD as described in the literature.[25,26] The NSD computational engine (http://jasheln.unm.edu/jasheln/content/nsd/NSDengine/docs_index.htm) was used for all decompositions.[46]

Results and Discussion

To find the effect of MCR on the hole size and flexibility of F430, we first determined the hole size and flexibility of cofactor F430 by itself, i.e., outside of MCR. Molecular mechanical calculations were used for this purpose. In molecular mechanics, it is common practice to drive a certain parameter, for example, a torsion angle, through a series of fixed values to obtain the strain energy as a function of the parameter. A curve of the strain energy versus the M−N bond distance can similarly be obtained by systematically increasing the ideal M−N distance in the force field and calculating the strain after each increase. The minimum of such a graph occurs at the ideal M−N bond length because the strain energy is at a minimum when the tetrapyrrole hole size perfectly matches the metal size.[40,41] The ideal M−N bond length therefore represents the metal-to-nitrogen distance of a hypothetical metal that would best fit the ligand in its lowest-energy conformation. Figure 2 shows the relative strain energy vs the imposed ideal metal-to-nitrogen distance curve obtained for six-coordinate cofactor F430, (orange

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Table 1. Ideal Ni−N Distances for Cofactor F430 in Its Different Epimeric States and Coordination Geometries

structure	metal coordination number	ideal M−N distance (Å)
F430 in protein (1MRO)	6	1.94−1.97
F430 in protein (1MRO)	4	1.94−1.97
F430	6	2.06
F430	5 (distal)	2.12
F430	5 (proximal)	2.02
F430	4	2.08
12,13 diepi-F430	6	2.04
12,13 diepi-F430	5 (distal)	2.06
12,13 diepi-F430	5 (proximal)	2.04
12,13 diepi-F430	4	2.02

triangles) and six-coordinate F430 in MCR (blue squares). According to the graph, the ideal average M−N distance of six-coordinate metal ion in cofactor F430 is 2.06 Å. The same procedure was carried out with cofactor F430 in the active site of MCR. The ideal metal−nitrogen distance is 1.97 Å when F430 is in MCR.

The hole scan of F430 and F430 in MCR shows that free F430 has a larger ideal Ni−N distance than it does when it is in the protein and that the tetrapyrrole is more rigid in the protein, i.e., deviations from the ideal Ni−N distance cause more rapid increases of the relative strain energy. This narrowing of the ideal metal distance caused by MCR imposing some rigidity upon F430 will affect the redox chemistry of MCR, since the nickel(II)/nickel(I) couple is associated with an increase in metal ion size.

Table 1 lists the ideal Ni−N distances for cofactor F430 in its different epimeric states and coordination geometries. Examination of the table and all the hole scans carried out reveals that the above-mentioned observations are true in all coordination geometries, free F430 always has a larger ideal Ni−N distance than F430 in MCR, and the flexibility of F430 is severely reduced in MCR.

To further examine the structural effects MCR imposes upon F430 as a function of its hole size, the nonplanar deformations of cofactor F430 were analyzed by NSD. This method determines the out-of-plane distortions in terms of the equivalent distortions along the lowest-frequency normal coordinates of the tetrapyrrole.[26] The predominant nonplanar deformations observed for the tetrahydrocorphinoid F430 were the B2u (saddled) and the B1u(ruffled) normal deformations. Figure 3 shows the degree of ruffling and saddling, as determined by NSD analysis, of all the six-coordinate nickel F430 (red) and six-coordinate nickel "F430 in MCR" (dark blue) structures used in the hole scans shown in Figure 2. It shows that the surrounding protein has a significant effect on the nonplanar deformations that the tetrahydrocorphinoid backbone of F430 undergoes in order to accommodate different metal sizes. In the absence of protein, F430 undergoes a significant ruffling deformation (>2 Å) to accommodate small metal ions (M−N < 1.90 Å), while increasing the ideal M−N distance results in a reduction in the amount of ruffling and an increase in the amount of saddling. Ruffling is also the predominant nonplanar deformation F430 undergoes when it is inside MCR. However, F430 is able to undergo significantly less ruffling in the

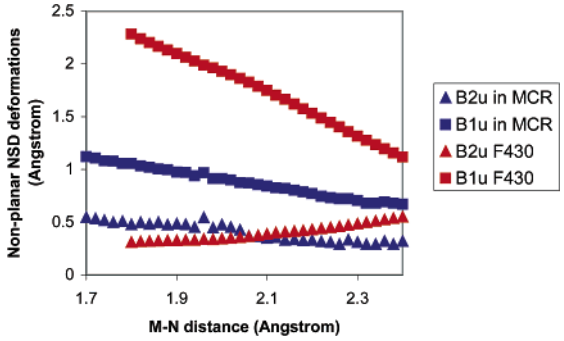

Figure 3. B2u(sad) and B1u(ruf) deformations of all the six-coordinate nickel F430 (red) and six-coordinate nickel F430 in MCR structures used in the hole scans shown in Figure 2. A 1 Å distortion means that the square root of the sum of the squares of the *z* displacements from the mean plane is equal to 1.[25]

Figure 4. Overlap of all structures, between 1.8 and 2.4 Å, that were used to generate Figure 2. F430 in MCR (left) undergoes less nonplanar deformations than F430 in solution (right).

protein, and increasing the metal size results in a decrease in both ruffling and saddling.

All the individual structures used to generate Figures 2 and 3 were overlapped. As expected, the overlaps show that F430 in MCR (Figure 4, left) is a flatter than F430 (Figure 4, right) and that it undergoes less nonplanar deformations. The main conformational differences occur in the C ring, which flips to accommodate larger metal ion sizes (see Figure 1). Figure 5 shows the cavity in MCR which contains cofactor F430. The cavity restricts the conformational freedom of F430, so it is not surprising that F430 undergoes less nonplanar deformations in MCR.

Since nonplanar tetrapyrroles tend not to bind axial ligands,[47,48] the MCR-imposed reduction in nonplanar deformations will presumably increase the likelihood of axial ligation in the MCR-bound F430. This is important because F430 is axially ligated in all active protein-bound forms, and axial ligand exchange occurs during the MCR catalytic reaction. We have therefore also examined the structural consequences of axial ligation.

A change from four-coordinate nickel to six-coordinate nickel has no major effect on the ideal metal−nitrogen distance of cofactor F430. However, Figure 6 shows that the coordination does affect how the tetrahydrocorphinoid backbone of F430 responds to a change in the metal size. In all complexes, we observed that free cofactor F430 undergoes more nonplanar deformations when it contains a four-coordinate metal ion than it does when it contains a six-

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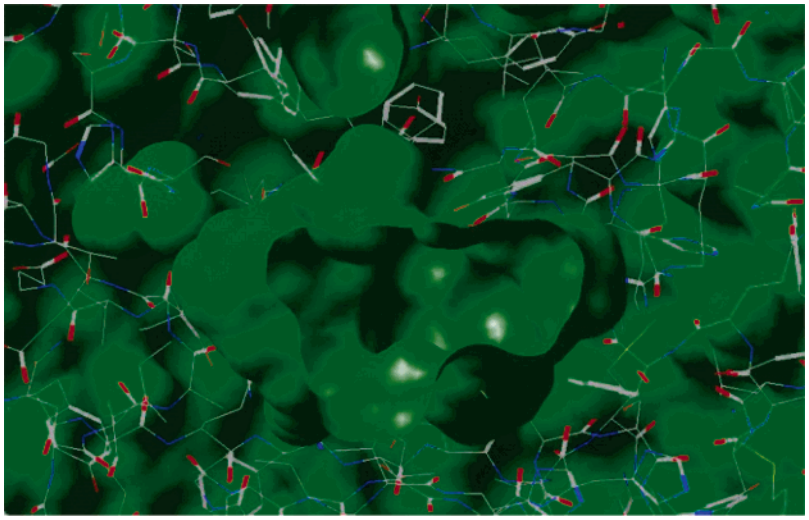

Figure 5. Molecular surface of one of the F430 cavities in MCR. The figure was obtained by removing F430 from the 1MRO MCR crystal structure and calculating the Connelly surface with a 1.2 Å probe radius.

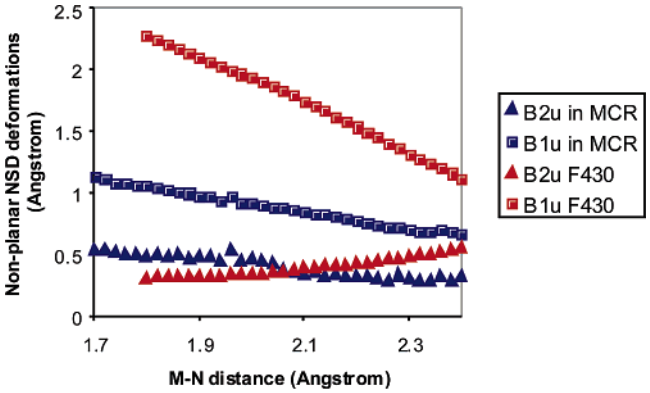

Figure 6. B2u(sad) and B1u(ruf) deformations of four-coordinate nickel F430 (red) and six-coordinate nickel F430 in MCR (blue) structures. A 1 Å distortion means that the square root of the sum of the squares of the *z* displacements from the mean plane is equal to 1.[25]

coordinate metal ion. Furthermore, in the absence of protein, F430 undergoes significantly more saddling than ruffling deformations to accommodate small metal ions (M−N < 1.90 Å); this is directly opposite to what was observed for six-coordinate F430. Increasing the ideal M−N distance in four-coordinate F430 results in a reduction in the amount of both the saddling and ruffling. In contrast to six-coordinate F430 in MCR, the nonplanar deformations of four-coordinate F430 in MCR do not vary much upon changing the size of the metal ion.

Conclusion

It has been suggested that proteins are able to enforce unstable geometries upon metal ions and their cofactors, thereby producing an entatic state that can increase the reactivity of the active site. Previously, we have shown that the protein matrix of MCR influences the chemistry at the nickel active site by preventing 12,13-diepi F430 from undergoing nonplanar deformations and therefore favoring F430 over the 12,13-diepimeric F430 form.[33] The strain imposed on 12,13-diepi F430 in the protein is so large that, although 88% of free F430 is found in the diepimeric form, none of the diepimeric form is found in MCR.

In this work, we have examined the effect MCR has on the hole size of F430 and we have shown that by restricting F430's nonplanar deformations MCR limits F430's hole-size flexibility. MCR also imposes a shorter ideal metal−nitrogen distance on the tetrahydrocorphinoid cofactor. This suggests that the protein environment (MCR) is responsible for tuning the chemistry of the active-site nickel ion.

It is known that the axial ligand affinities are affected by the degree of nonplanarity present in tetrapyrroles[49] and that the hole size affects the redox potential due to the fact that nickel(I) is significantly larger than nickel(II). This suggests that the protein environment (MCR) is capable of tuning the chemistry (redox potentials and axial ligand affinity) of the active-site nickel ion and that computational studies such as this might be able to locate residues that can be mutated to moderate the extent of the nonplanar deformations found in F430, thereby changing its reactivity.

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