Mitochondial Amidoxime Reducing Component (mARC)

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1 Introduction

Molybdenum is a trace element found in almost all forms of life on Earth. Previously there were only three known molybdenum-containing enzyme families: sulphite oxydases (SO), xantine oxidases (XO), and aldehyde oxidases. By 2006, a new Mo-containing protein was discovered, the Mitochondrial Amidoxime Reducing Component, with an unique reductive function.[1]

Mammalian genome studies have shown the presence of two main mARC containing genes: *MARC1* and *MARC2*. These genes encode two variations of the enzyme (mARC1 and mARC2).[1] Some structural divergences between both variations have been found, which have affected their catalytic activities. The human equivalents of these enzymes have been named hmARC1 and hmARC2.[1]

Mitochondrial amidoxime reducing components are found in almost all cells in an organism. Yet, a maximum expression of the gene has been observed in adipose cells, specifically in the liver, intestine, kidney, pancreas and thyroid gland cells. The enzymes are located in the external part of the mitochondrial membrane. The active site is exposed to the cytoplasm. Although, some samples of mARC proteins can be found in peroxisome membranes.[1] It may have the purpose of reducing unwanted by-products in the organelle.

1.1 Biological Function

As its name indicates, mARC enzyme's main purpose is reducing amidoximes in the cell. These substances are commonly synthesized as by-products of oxidative metabolisms. For instance, sulfonamide sulfamethoxazole is naturally metabolized by the cytochrome P450 complex (Figure 1C). This compound may have toxic and mutagenic repercussions in the body. Thus, mARC enzymes catalyze this compound to a reduced and non toxic product.[1]

Mitochondrial amidoxime reducing components can reduce a wide range of N-oxidaded substances. For instance, gut microbiota transform compounds such as l-carnitine, betaine, choline, etc. to triethylamine, which can then be oxidised to triethylamine nitroxide (Figure 1D). This compound may cause cardiovascular diseases. Yet, hmARC1 enzymes may reduce it back to TMA, which can then be discarded by the body.[2]

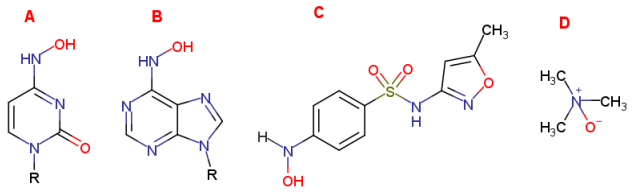

Figure 1: Exemplars of toxic and mutagenic metabolites that can be reduced by mARC. (A) N^4-hydroxycytosine, (B) N^6-hydroxyadenine, (C) sulfonamide sulfamethoxazole, (D) triethylamine nitroxide.[1 2]

2 Structure

Experimental studies show that mARC enzymes are located adjacent to a cytochrome b_5. This proteins transports electrons coming from a NADH-cytochrome b_5 reductase. This electron transport chain can be disrupted by eliminating one of these proteins of the system. then, its reductive activity will cease.[1 2]

The crystalline structure of mARC enzymes wasn't known until Kubitza *et al.* elucidated the structure by X-ray diffraction. The gene of the protein hmARC1-T4L was expressed in a *Escherichia coli*, where it was then isolated and crystallised (Figure 2).[3]

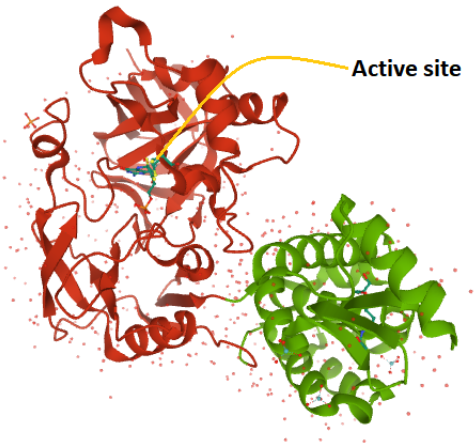

Figure 2: Crystalline structure of the protein mARC1. The location of the active site is indicated. **PDB: 6FW2**.[3]

The protein is composed of two sub-units. The majority of its secondary structure derives from β-sheets. Yet, some α-helices and other non-repetitive structures can be found.[3]

Structural differences between mARC1 and mARC2 have been found. Some divergences have been observed at some amino-acids near the active site. For instance, S271 residue from mARC1 is substituted by a proline in mARC2. Also, H152 is changed to a phenylalanine.[3] It's suspected that this slight variations affect the reactivity of mARC2 (Figure 3). For instance, mARC1 has a large reactive versatility. It can reduce nitrogen species with higher oxidation states, such as nitrites and nitric oxide. On the other hand, mARC2 enzymes lack this properties.[2 4]

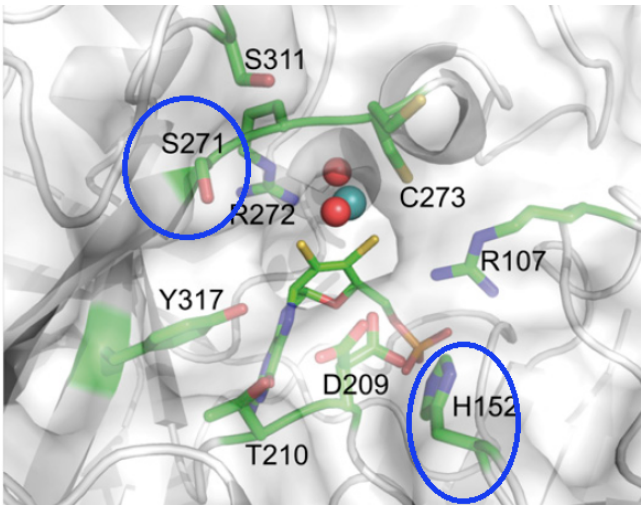

Figure 3: Crystalline structure of mARC1 enzyme near its active site. The circled residues (H152 y S271) change in mARC2.[3]

The protein contains only one co-factor, which is a penta-coordinated molybdenum metallic center. It is known as the *Moco* site. Electronic paramagnetic resonance spectroscopy shows that molybdenum has one electron in its *d* orbitals before the catalysis. It can thus be confirmed an oxidation state of Mo (IV).[4]

The metallic center is coordinated to a pyranopterin dithiolene (pdt) ligand, as well as a cystein and two oxygen-based ligands, in which one of those is axially bound. The chemical nature of these oxygen ligands has been widely debated. A triple bond M≡O, along with an aqua ligand was initially predicted. Other structures have been predicted, such as an axial double bond with a hydroxyl ligand.[4] X-ray studies by Kubitza *et al.* confirm that the conformation of M=O has a higher probability due to its bond distances OH^-.[3]

It was assumed that the molybdenum co-factor had a square based pyramid geometry. Yet, X-ray elucidation studies show otherwise. Its structure shows multiple intermolecular forces between the pdt ligand and multiple amino-acids. These interaction fixes the chelate in a specific site, which distorts the co-factor's symmetry (Figure 4). Therefore, the complex has an intermediate structure between a trigonal bi-pyramidal and a square based pyramid.[3]

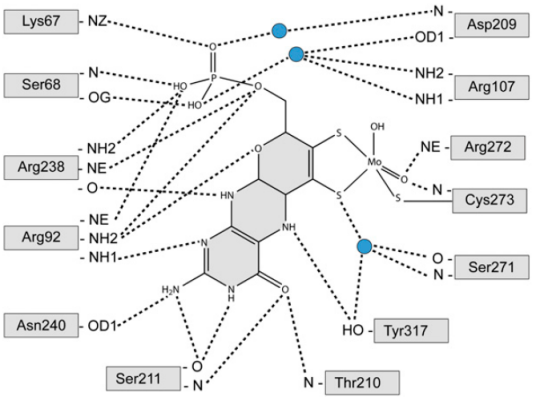

Figure 4: Molecular structure of the molybdenum cofactor. Interactions between the pdt ligand and other amino-acids are shown.[3]

3 Mechanism

The mitochondrial amidoxime reducing component has the catalytic function of reducing N-oxidized species.[4 1] Thus, an effective electron transport chain is needed. The process begins with an NADH oxidation at the NADH-cytocrhome b_5 reductase. This protein transports its electrons to the cytochrome b_5, which are then transferred to the mARC. This electron reduces the metallic center to Mo(IV) (Figure 5). This reduced state carries out the catalysis.[1 5]

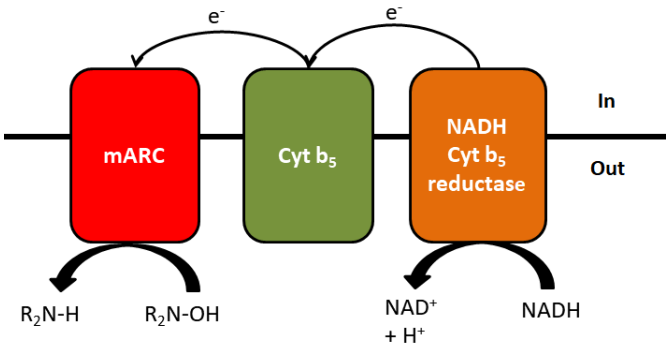

Figure 5: Schematic view of the electron transport chain to the mARC enzymes in the external mytochondrial membrane.[1 2]

Two main mechanisms for the reduction have been proposed. The first mechanism is based on the triple bonded M≡O oxo ligand. The metallic center begins with an oxidation state of (IV). The substrate substitutes the hydroxyl ligand, which is detached as water. The electrons stored at the *d* orbitals are transferred to the substrate, which is detached. Then, a radical rupture occurs at the axial oxygen. Therefore, the molybdenum gets an oxidation state of (V). Electrons from the ETC return the co-factor to its initial state (Figure 6).[4]

The second proposed mechanism is based on the structural model elucidated by X-ray diffraction. Molybdenum begins with an (IV) oxidation state, yet its oxo ligand is doubly bound. The substrate enters and it's reduced by the same mechanism. Although, no radical rupture occurs after

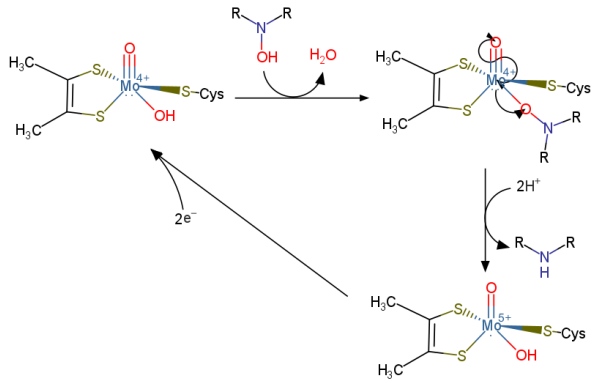

Figure 6: First mechanism of reaction proposed for the active site of the mARC enzyme.[4]

the electronic donation. Thus, the molybdenum acquires an oxidation state of (VI) (Figure 7).[3]

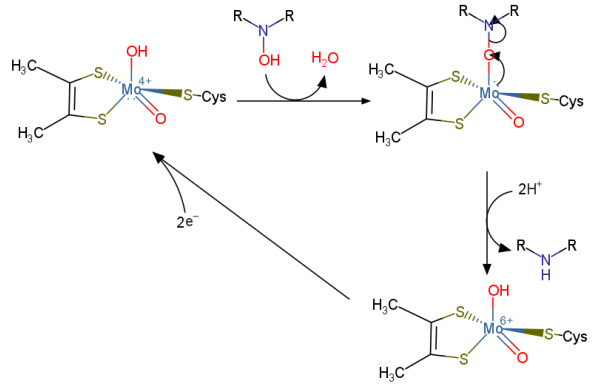

Figure 7: Second mechanism of reaction proposed for the active site of the mARC enzyme.[3]

The mechanism shown in Figure 6 is thermodynamically favoured, as the molybdenum reaches a stable electronic state of d^0. Yet, it doesn't consider the steric restrictions previously discussed 4. The mechanism proposed by Kubitza *et al.* does take into account these considerations (Figure 7).[3]

4 Applications

Mitochondrial amidoxime reducing component is widely investigated in the pharmaceutic industry.[5] Its reductive versatility for N-oxides has generated an overall interest for its potential use as a pro-drug reaction chamber. These types of medicines are consumed in an inactive form, yet are metabolized to its activated state. This strategy can reduce the toxicity of a drug when consuming. Therefore, larger doses can be prescribed. Also, its specificity may increase. For instance, a common functional group found in medical drugs. Although, its alkalinity allows only the consumption of small doses. If it's fabricated as an amidoxime, its pH decreases, and it can be then reduced by mARC enzymes in the organism.[1]

4.1 Recent Studies

mARC's reductive capability depends on the substrate's composition as well. Therefore, it won't react with every variation of N-oxidised compounds.[5] Hence, it has recently been studied quick methods of testing its substrate-dependent reactivity (specifically focused on medical drugs). The enzyme's efficiency has been proven by analyzing its NADH consumption. This activity directly indicates metabolic activity on the proteic complex. The products are then evaluated by gas chromatography coupled with mass spectrometry to corroborate if the substrate got successfully reduced. With this method, amidoxime pro-drug evaluation would be considerably expedited.[5]

References

[1] Ott, G.; Havemeyer, A.; Clement, B. The mammalian molybdenum enzymes of mARC. *JBIC Journal of Biological Inorganic Chemistry* **2015**, *20*, 265–275.

[2] Schneider, J.; Girreser, U.; Havemeyer, A.; Bittner, F.; Clement, B. Detoxification of Trimethylamine *N* -Oxide by the Mitochondrial Amidoxime Reducing Component mARC. *Chemical Research in Toxicology* **2018**, *31*, 447–453.

[3] Kubitza, C.; Bittner, F.; Ginsel, C.; Havemeyer, A.; Clement, B.; Scheidig, A. J. Crystal structure of human mARC1 reveals its exceptional position among eukaryotic molybdenum enzymes. *Proceedings of the National Academy of Sciences* **2018**, *115*, 11958–11963.

[4] Yang, J.; Giles, L. J.; Ruppelt, C.; Mendel, R. R.; Bittner, F.; Kirk, M. L. Oxyl and Hydroxyl Radical Transfer in Mitochondrial Amidoxime Reducing Component-Catalyzed Nitrite Reduction. *Journal of the American Chemical Society* **2015**, *137*, 5276–5279.

[5] Indorf, P.; Kubitza, C.; Scheidig, A. J.; Kunze, T.; Clement, B. Drug Metabolism by the Mitochondrial Amidoxime Reducing Component (mARC): Rapid Assay and Identification of New Substrates. *Journal of Medicinal Chemistry* **2020**, *63*, 6538–6546.