Understanding the Molecular Basis of Multiple Mitochondrial Dysfunctions Syndrome 1 (MMDS1)—Impact of a Disease-Causing Gly208Cys Substitution on Structure and Activity of NFU1 in the Fe/S Cluster Biosynthetic Pathway

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Abstract

Iron–sulfur (Fe/S)-cluster-containing proteins constitute one of the largest protein classes, with varied functions that include electron transport, regulation of gene expression, substrate binding and activation, and radical generation. Consequently, the biosynthetic machinery for Fe/S clusters is evolutionarily conserved, and mutations in a variety of putative intermediate Fe/S cluster scaffold proteins can cause disease states, including multiple mitochondrial dysfunctions syndrome (MMDS), sideroblastic anemia, and mitochondrial encephalomyopathy. Herein, we have characterized the impact of defects occurring in the MMDS1 disease state that result from a point mutation (Gly208Cys) near the active site of NFU1, an Fe/S scaffold protein, via an *in vitro* investigation into the structural and functional consequences. Analysis of protein stability and oligomeric state demonstrates that the mutant increases the propensity to dimerize and perturbs the secondary structure composition. These changes appear to underlie the severely decreased ability of mutant NFU1 to accept an Fe/S cluster from physiologically relevant sources. Therefore, the point mutation on NFU1 impairs downstream cluster trafficking and results in the disease phenotype, because there does not appear to be an alternative *in vivo* reconstitution path, most likely due to greater protein oligomerization from a minor structural change.

Introduction

Mitochondria are complex eukaryotic organelles that serve as the site of aerobic cellular metabolism and energy production via oxidative phosphorylation. Furthermore, many important and diverse pathways for the production of essential biological cofactors are localized to the mitochondria [11–13]. Given the inherent structural and functional complexity of the mitochondria, combined with the necessary biosynthetic processes that occur there, mitochondrial defects can cause a wide variety of severe and generally untreatable disorders [11,15]. Multiple mitochondrial dysfunctions syndrome (MMDS) constitutes a class of typically fatal diseases that result from the severe impairment of various metabolic pathways and energy production as a consequence of single nucleotide genetic mutations [16,17]. Symptoms of MMDS include hypotonia, respiratory insufficiency, hyperglycinemia, encephalopathy, neurological regression, and failure to thrive, indicative of decreased functional actions of mitochondrial respiratory complexes [18–20]. Additional symptoms of MMDS are impaired function of lipoic acid-dependent enzymes, such as pyruvate dehydrogenase and protein H of the glycine cleavage system [15,16,21]. Due to the severity and extent of symptoms described above, MMDS is typically fatal during perinatal stages [16];

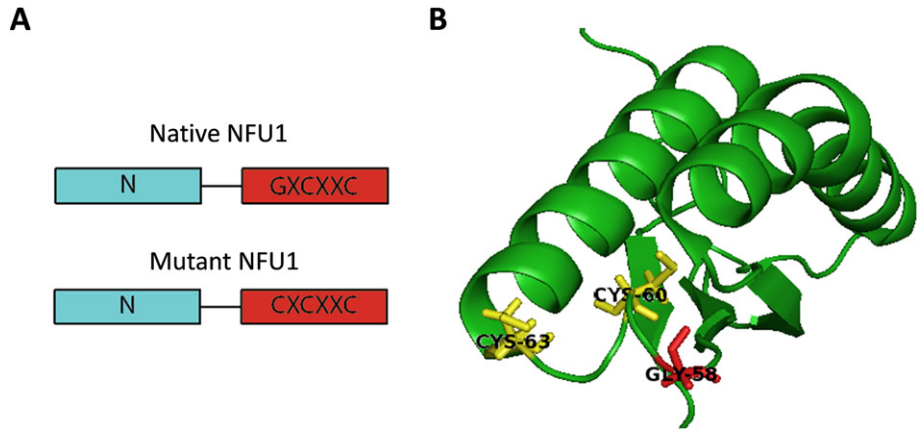

Fig. 1. (A) A representation of the two-domain composition of the native and mutant NFU1 proteins, with the N-terminal domain in blue and the C-terminal domain in red. NFU1 features the functional CXXC Fe/S cluster binding motif in its C-terminal domain. This pattern is altered in the mutant protein with the mutation of a nearby glycine residue at position 208 to cysteine, which gives a CXCXXC motif. (B) Solution NMR structure of human C-terminal domain of the NFU1 protein (PDB ID: 2M5O) with the cluster binding cysteines shown in yellow [5]. The glycine at position 208 in the full-length protein (58 above), which is mutated to a cysteine in MMDS1, is colored red. Numbering is consistent with the C-terminal construct used in the structure determination.

however, some patients have lived until the age of two, at which point the symptoms culminated in death [17,18,20,21].

Interestingly, all four types of MMDS so far identified are associated with genes that code for metalloproteins, specifically those involved in the biosynthesis of iron–sulfur (Fe/S) clusters: IBA57, IscA2, BOLA3, or NFU1 [16,17,22–26]. Further inspection of the defects caused by these Fe/S cluster proteins has revealed a specific impairment of downstream [4Fe-4S]-cluster-containing proteins; by contrast, [2Fe-2S]-cluster-containing proteins and cytosolic Fe/S cluster proteins appear unaffected [15,17,22]. All four of the identified proteins have been implicated in Fe/S cluster biosynthetic pathways as mediators of cluster transfer and delivery. Fe/S clusters are highly conserved inorganic prosthetic groups that are present across all kingdoms of life and play diverse roles in the cell, including electron transfer, regulation of gene expression, and disulfide reduction [27,28].

Herein, we have focused on one of the aforementioned Fe/S proteins, NFU1. Human NFU1 consists of two domains, an N- and a C-terminal domain [4,29–31], where the latter contains a highly conserved CXXC motif [31] that identifies this domain as the Nfu domain and suggests a role in Fe/S cluster binding, assembly, and transport, based on comparison to homologous proteins and the high level of sequence conservation [32–36]. The thermodynamic stability of NFU1 has been studied in depth to reveal that the isolated C-terminal domain of human NFU1 exhibits characteristics of a molten globular state [4,30], while the isolated N-terminal domain demonstrates a highly ordered structure. However, when the two domains come together, the overall protein maintains a relatively well-folded structure, suggesting a requirement for the unique N-terminal domain of the human protein to provide structural stabilization to serve a potential functional role [4,30] that may include a protein oligomerization surface or a binding site for chaperones, such as Hsc20 [5,37]. Structure–function characteristics of human NFU1 remain unclear, because this protein has been implicated in a variety of cellular roles. NFU1 is also known as the HIRA-interacting protein, where HIRA is the histone cell cycle regulation homolog A [38]. Furthermore, it exhibits thioredoxin-like activity in the apo form [29,30], assembles and transfers a [2Fe-2S] cluster [1], assembles a [4Fe-4S] cluster [31,39], and may transfer cluster to apo aconitase [5]. Homologs of human NFU1 have been implicated in similar roles, with [2Fe-2S] [33,34,36,40] and [4Fe-4S] [32,33,35,41] cluster transfer, suggesting that both functionalities are possible and potentially physiologically relevant. Most recently, NFU1 has been implicated in the disease phenotype of MMDS1 attributed to a c.622G>T missense mutation located in the gene encoding the protein NFU1 [17,21]. This introduces a p.Gly208Cys missense mutation in the protein close to the Fe/S cluster binding motif (Fig. 1) that alters the region around the cluster binding motif from GXCXXC to CXCXXC.

In connection with the MMDS1 disease state, human NFU1 has been proposed to be involved in the maturation of the Fe/S clusters on the [4Fe-4S] target proteins lipoate synthase and succinate dehydrogenase (SDH) [17,21,39]. In patients with MMDS1, laboratory tests have demonstrated that the level of human NFU1 protein is not diminished [17,21,42], but the activity levels of lipoate synthase and SDH are significantly impaired, while other [4Fe-4S]