Structure/Function Relations in *AIFM1* Variants Associated with Neurodegenerative Disorders

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Abstract

The X-linked *AIFM1* gene encodes mitochondrial apoptosis-inducing factor (AIF), an FAD-containing and NADH-specific oxidoreductase critically important for energy metabolism and execution of the caspase-independent cell death pathway. Several recently identified mutations in human *AIFM1* lead to neurodegenerative disorders varying in severity and onset time. This study was undertaken to structurally and functionally characterize four pathologic variants of human AIF: V243L, G262S, G308E, and G338E. A strong correlation between the mutational effects on the AIF function and clinical phenotype was observed only for the G308E aberration, drastically damaging both the redox properties of AIF and mitochondrial respiration. In contrast, only minimal or mild changes were detected in the structure/function of AIF V243L and G338E, respectively, indicating that a marked decrease in their cellular expression likely triggers the disease. Alterations in the structure and redox activity of AIF G262S, on the other hand, were more severe than could be predicted based on the clinical phenotype. Together, the results of this and previous studies allow to conclude that the phenotypic variability and severity of the *AIFM1*-related disorders depend on which AIF feature is predominantly affected (i.e., cellular production level, structure, redox or apoptogenic function) and to what extent. Only a drastic decrease in the expression level or/and redox activity of AIF tends to cause an early and severe neurodegeneration, whereas less pronounced changes in the AIF properties could lead to a broad range of slowly progressive neurological disorders.

Introduction

The human *AIFM1* gene is located on the X chromosome and encodes apoptosis-inducing factor (AIF), a mitochondrial FAD-containing protein [1]. AIF is synthesized in the cytoplasm and, upon maturation and import to the mitochondrial intermembrane space, N-terminally attaches to the inner mitochondrial membrane. After accepting two electrons from NADH in a form of a hydride ion, AIF undergoes dimerization and a conformational change, resulting in the formation of an air-stable $FADH^{\bullet}$–NAD^{+} charge-transfer complex (CTC) [2–4]. AIF was initially suggested to function *in vivo* as a superoxide-producing NADH oxidase [5] and, most recently, as a rotenone-sensitive NADH:ubiquinone oxidoreductase [6]. Alternatively, based on the unique structural properties of the dimeric CTC form and its resistance to oxidation, AIF was proposed to operate as a NAD^{+}(H)-sensor capable of regulating mitochondrial morphology and function through a specific redox activity or/and protein–protein interactions in the intermembrane space [2,3,7]. Indeed, two recent studies [8,9] provided the first evidence that AIF modulates the oxidative phosphorylation (OXPHOS) process through direct interaction and functional regulation of Mia40 (mitochondrial intermembrane space import and assembly 40, also known as CHCHD4), a component of the disulfide relay system playing a central role in the biogenesis and assembly of the respiratory chain complexes I and IV [10]. In addition to its vital role in mitochondrial respiration, AIF is also a key player in a caspase-independent cell death pathway [1]. Upon an apoptotic insult and proteolysis of the membrane tether, AIF translocates to the nucleus and, in cooperation with other proteins, promotes chromatin condensation and large-scale DNA degradation (for review, see Ref. [7]).

Studies on isolated cells and animal models showed that severe defects in the redox function or production

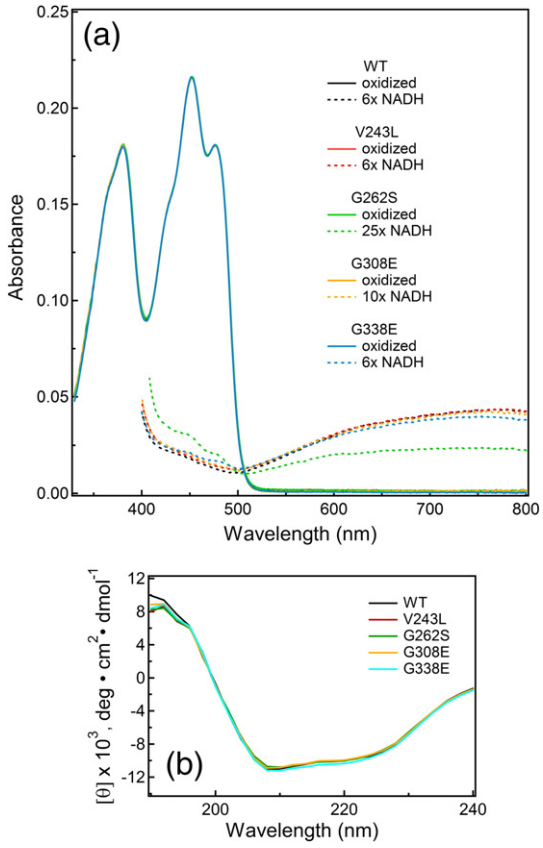

Fig. 1. (a) Absorbance spectra of the oxidized (solid lines) and NADH-reduced (dotted lines) WT AIF and mutants. A various excess of NADH (indicated) was used to fully reduce FAD. (b) Far-UV circular dichroism spectra of the WT and mutant AIF.

of AIF could lead to mitochondrial fragmentation and respiratory defects (for review, see Ref. [11]). To date, a number of pathologic mutations in human *AIFM1* have been identified [12–18], among which only two, ΔR201 and E493V, were investigated on a molecular level. Deletion of Arg201 was found to perturb the protein folding and incorporation of FAD, affecting both the redox and apoptogenic properties of AIF and leading to a severe and early-onset encephalomyopathy [12,13]. In contrast, the E493V mutation caused only modest structural changes but enhanced the apoptogenic properties of AIF and, as a result, led to a slowly progressive neuronal damage [13].

The goal of this study was to investigate the impact of four other pathologic mutations, V243L, G262S, G308E, and G338E [15–18], on the structure and function of human AIF. Elucidation of how changes in the gene and encoded protein relate to the phenotype is one of the central problems in biomedical research, because this knowledge enables to distinguish genetic polymorphism from disease-causing mutations and find best approaches for the disease treatment and genetic manipulation. Data reported here not only provide deeper insights into the structure/function relationships in AIF but also clarify the pathologic mechanism of *AIFM1* aberrations.

Results

Expression and folding of the AIF mutants

Production of AIF V243L, G262S, and G338E in human cells was reported to be markedly reduced [15,18]. In *Escherichia coli*, however, all mutants were expressed at high levels with fully incorporated FAD. Similar to the wild type (WT) AIF, all variants except G262S remained partially reduced during purification, forming an air-stable and blue-colored $FADH^{\bullet}$–NAD^{+} CTC. In contrast, AIF G262S was oxidized immediately after separation from the bacterial cell lysate, which was the first indication of its perturbed redox properties. The oxidized proteins had absorbance and circular dichroism spectra nearly identical to those of WT (Fig. 1). This and similar flavin extinction coefficients (Table 1) indicate that none of the mutations affects protein folding and incorporation of FAD.

Mutational effects on the equilibrium NADH binding, NADH-to-FAD hydride transfer kinetics, CTC stability, and redox activities of AIF

Equilibrium titrations with NADH

Similar to WT, none of the investigated mutants stabilized the 1-electron reduced FAD semiquinone during anaerobic titrations (Fig. 2). AIF V243L and G338E required only a slight NADH excess to convert to the 2-electron reduced CTC form (Fig. 2b and c). On the contrary, a large excess of the reductant was needed to fully reduce AIF G262S (Fig. 2d), which reacted with NADH considerably slower and produced CTC with a ~2-fold weaker long-wavelength absorption ($\varepsilon^{CTC}_{780nm}$ of 1.6 *versus* 2.9 $mM^{-1}\,cm^{-1}$ for WT; Fig. 1a). Moreover, the spectral dissociation constant estimated for the AIF–G262S–NADH complex (K_s^{NADH}, a measure of the binding affinity) was almost 300-fold higher relative to that for WT (Table 1). One contributing factor for such a drastic change could be a 36-mV decrease in the redox potential of AIF G262S ($E_{m,8}$ of −389 *versus* −353 mV for WT and −350 mV for NADH), lowering the driving force for the NADH-to-FAD hydride transfer (HT), due to which higher NADH concentrations are required to shift equilibrium toward the fully reduced FAD. The G308E mutation had no effect on the AIF redox potential, but reduction of this variant with NADH proceeded extremely slowly. Due to time limitations, K_s^{NADH} for AIF G308E was derived from a composite titration plot