The G308E variant of the apoptosis inducing factor, responsible of a rare encephalopathy, is hampered in NAD^+/H binding

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The apoptosis inducing factor (AIF) is a highly conserved mitochondrial flavoprotein known to play two opposite roles in eukaryotic cells: in mitochondria it is required for efficient oxidative phosphorylation (OXPHOS), while, when released into the cytoplasm, it triggers caspase-independent apoptosis (*1*). The mechanism of AIF-induced apoptosis was extensively investigated, whereas its mitochondrial role is poorly understood. There are many evidences of AIF importance for mitochondrial correct morphology and functions and recently the discovery of its direct interaction with CHCHD4, a key regulator of respiratory complexes subunits import and folding in mitochondria, was reported (*2*). A unique feature of AIF, probably pivotal for its vital function, is its ability to form a tight, air-stable charge-transfer (CT) complex with NAD^+ and undergo dimerization. Although some aspects of AIF interaction with NAD^+/H have been analyzed, its precise mechanism is not fully understood. We investigated the effect of the pathogenic G308E replacement, associated with OXPHOS defect and neurodegeneration (*3*), to understand how it could alter AIF properties at the molecular level. To do so, we analysed how the wild type and the G307E forms of murine AIF interact with NAD^+/H and nicotinamide mononucleotide (NMN^+/H), finding that the pathogenic replacement resulted in a dramatic and specific decrease of the rate for CT complex formation and consequent protein dimerization only in the case of the physiological ligand. Our results demonstrates that the adenylate moiety of NAD^+/H is crucial for the ligand binding process and that the G307E replacement causes an alteration of the adenylate-binding site of AIF that drastically decreases the affinity for and the association rate of the ligand. In addition, we shed new light on the mechanism of the dimerization process, demonstrating that FAD reduction rather than NAD^+/H binding initiates the conformational rearrangement of AIF that leads to quaternary structure transitions.

Taken together, our results contribute to defining how AIF works at the molecular level in binding NAD^+/H and undergoing dimerization, and also point out that the G308E replacement, responsible of a rare neurodegenerative disease, has the selective effect of slowing down the formation of AIF dimeric CT complex.

1. Sevrioukova (2011) *Antioxid Redox Signal*, **14**: 2545-2579
2. Hangen *et al* (2015) *Molecular Cell*, **58**: 1-14
3. Berger *et al* (2011) *Mol Genet Metab*, **104**: 517-520