LHON/MELAS overlap mutation in ND1 subunit of mitochondrial complex I affects ubiquinone binding as revealed by modeling in *Escherichia coli* NDH-1

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ABSTRACT

Defects in complex I due to mutations in mitochondrial DNA are associated with clinical features ranging from single organ manifestation like Leber hereditary optic neuropathy (LHON) to multiorgan disorders like mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS) syndrome. Specific mutations cause overlap syndromes combining several phenotypes, but the mechanisms of their biochemical effects are largely unknown. The m.3376G>A transition leading to p.E24K substitution in ND1 with LHON/MELAS phenotype was modeled here in a homologous position (NuoH-E36K) in the *Escherichia coli* enzyme and it almost totally abolished complex I activity. The more conservative mutation NuoH-E36Q resulted in higher apparent K_m for ubiquinone and diminished inhibitor sensitivity. A NuoH homolog of the m.3865A>G transition, which has been found concomitantly in the overlap syndrome patient with the m.3376G>A, had only a minor effect. Consequences of a primary LHON-mutation m.3460G>A affecting the same extramembrane loop as the m.3376G>A substitution were also studied in the *E. coli* model and were found to be mild. The results indicate that the overlap syndrome-associated m.3376G>A transition in *MTND1* is the pathogenic mutation and m.3865A>G transition has minor, if any, effect on presentation of the disease. The kinetic effects of the NuoH-E36Q mutation suggest its proximity to the putative ubiquinone binding domain in 49 kD/PSST subunits. In all, m.3376G>A perturbs ubiquinone binding, a phenomenon found in LHON, and decreases the activity of fully assembled complex I as in MELAS.

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1. Introduction

NADH-ubiquinone oxidoreductase (Complex I, EC 1.6.5.3) is by far the most complicated enzyme of the mitochondrial respiratory chain. It is composed of 45 different polypeptides and a set of redox centers

resulting in a total molecular mass of about 1000 kDa in mammals [1]. Seven of the complex I subunits (ND1–ND6 and ND4L) are encoded in mitochondrial genome (mtDNA) while the rest of the genes are located in the nucleus. Fourteen of the subunits, including all the NDs, have their homologs in NDH-1, the bacterial counterparts of the enzyme, and are considered to comprise the catalytic core of complex I (for a review, see [2]).

Regardless of increasing knowledge of the structure of complex I [3–6], the number and exact position of ubiquinone binding sites are still ambiguous. The iron–sulphur cluster N2 in the PSST subunit is thought to be the final electron donor to ubiquinone (UQ). Based on extensive mutational analysis of the PSST and 49 kD subunit homologs in *Yarrowia lipolytica* it has been demonstrated that many conserved residues lining the cavity between these peptides in the proximity of N2 are critical for the catalytic activity and inhibitor binding. It has therefore been suggested that a UQ binding domain is located within this pocket, although apart from Tyr-144 mutations in 49 kD subunit, most mutations with appreciable changes in inhibitor sensitivity affect very little the apparent K_m value for UQ [7–11]. A second puzzling

feature of UQ and inhibitor binding is that binding equilibrium data indicate only partially competitive binding for classical, quinone site-binding inhibitors like piericidin A. Some others, like rotenone, show a more mixed (non-competitive) type of inhibition [12], although all these inhibitors are regarded to bind to separate partially overlapping sites in the same domain [13].

ND1 as one of the core subunits in the membrane domain of complex I is apparently involved in attaching the hydrophilic part of the enzyme to the membrane [6,14]. Based on the locations of the helices of the membrane arm of the holoenzyme in *Thermus thermophilus*, an essential role has been proposed for ND1 in coupling the electron and proton transfer reactions of complex I [6]. Some evidence points to its involvement in UQ-binding, too. Structure of the membranous domain of *Escherichia coli* and *T. thermophilus* NDH-1 in combination with previous biochemical evidence has revealed a putative ubiquinone entry channel or binding site in NuoH/Nqo8, which are homologs of the mitochondrial ND1 subunit [6,15].

Complex I dysfunction is a common cause of mitochondrial disorders such as mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes syndrome (MELAS) and Leber hereditary optic neuropathy (LHON). At least 33 disease-associated substitutions have been reported so far in the *MTND1* gene (Mitomap, available from http://mitomap.org/MITOMAP, on April 14th 2011) emphasizing the importance of ND1 in complex I function. Similarly to the common m.3243A>G MELAS mutation [16–18], a decrease in amount of active complex I or its stability has been reported in transmitochondrial cybrid cell lines derived from patient fibroblasts and in bacterial models displaying ND1-subunit MELAS mutations [19–21]. In the case of ND1-affecting m.3460G>A mutation, one of the primary mutations associated with non-syndromic LHON, the in vitro electron transfer activity of the enzyme complex is strongly reduced and resistance to rotenone has been documented (for a review, see e.g. [22]). *MTND1* is a target for less common disease-associated mtDNA mutations as well. A potentially pathogenic, heteroplasmic m.3376G>A mutation in *MTND1* has been discovered in a patient suffering from the LHON/MELAS overlap syndrome [23]. The proband carried also an m.3865A>G transition affecting the same subunit, but it was not considered to be pathogenic since it was also present in the unaffected mother of the proband. In skeletal muscle, the citrate synthase-

normalized complex I activity was 36% of control [23]. Effects of this mutation on complex I have not been scrutinized any further. Interestingly, the m.3376G>A transition results in replacement of the highly conserved glutamate residue (Glu-24) by lysine in ND1 in an extramembranous loop, which is also a target of the common m.3460G>A LHON mutation as well as many other disease-associated substitutions (see Fig. 1).

The m.3376G>A mutation does not fulfill all the commonly accepted criteria for a pathogenic mtDNA mutation [24] and also the role of the m.3865A>G transition in the formation of the disease phenotype has remained unproven. We show here by employing an *E. coli* model that m.3376G>A mutation is clearly pathogenic, whereas m.3865A>G has only minor, if any, effect. Interestingly, m.3376G>A decreases enzyme amount similarly to other MELAS mutations affecting the same gene [19] and also disturbs the ubiquinone binding domain, which has been previously shown to be associated with LHON mutations [22,25,26]. To our knowledge, this is the first time the biochemical consequences of an mtDNA mutation associated with an LHON/MELAS overlap syndrome is analyzed on a stable, bacterial application framework. Because of the relative paucity of the mtDNA mutations, their metabolic link to human disease is difficult to confirm on basis of biopsy samples. Therefore, their investigation by means of site-directed mutagenesis in bacterial models is gaining increasing practice, exemplified by studies on LHON, [25–28] and MELAS [19]. Although limited to the structural genes of the core subunits and to the effects at enzymatic level, a bacterial model for an mtDNA mutation enables introduction of mutations not occurring naturally, to better analyze the consequences of a single amino acid substitution.

2. Materials and methods

2.1. Chemicals

Decylubiquinone (DB), ubiquinone-1 (Q_1), ubiquinone-2 (Q_2), NADH, deamino-NADH (d-NADH), DL-malate, N-vanillylnonanamide (VNA), soya bean phospholipids (Asolectin) and p-Nitro-Blue tetrazolium chloride (NBT) were from Sigma. Hexaammineruthenium (HAR) was purchased from Aldrich, Hepes and Mes from AppliChem, and
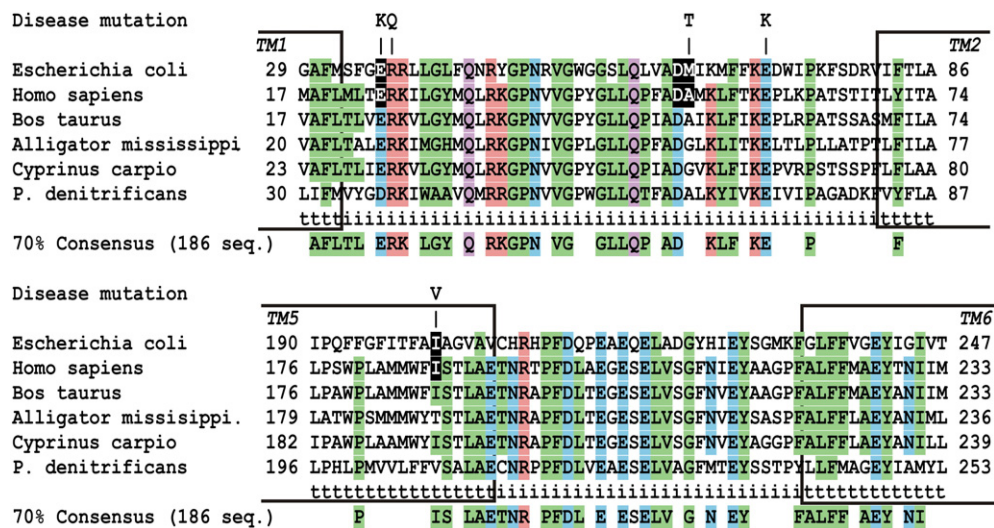

Fig. 1. The first and the third inside loops of human ND1 and *E. coli* NuoH with parts of bordering transmembrane helices 1 and 2, 5 and 6. Disease-associated mtDNA mutations and the corresponding amino acid changes p.E24K (m.3376G>A) [23], p.R25Q (m.3380G>A) [53], p.Y30H (m.3394T>C) [54,55], p.M31V (m.3397A>G) [56], p.A52T (m.3460G>A) [57], p.E59K (m.3481G>A) [21,58] and p.I187V (m.3865A>G) [23] are indicated. The residues undergoing site-directed mutagenesis are shown in white font on black background. An alignment of homologous complex I subunits from human (GenBank ID: AEO90223.1), bovine (GenBank ID: ADF49509.1), carp (NCBI ID: NP_007082.1), alligator (UniProt ID: O47868), *E. coli* (GenBank: AAC75342.1) and *P. denitrificans* (GenBank ID: AAA25592.1) was performed by means of ClustalW, and the transmembrane topology by means of the TMHMM program [40]. "t" denotes transmembrane helix and "i" inside loop. The 70% consensus sequence was compiled by the PredictProt server from an alignment of 186 homologous sequences.