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Tracing the tail of ubiquinone in mitochondrial complex I ☆

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ABSTRACT

Mitochondrial complex I (proton pumping NADH:ubiquinone oxidoreductase) is the largest and most complicated component of the respiratory electron transfer chain. Despite its central role in biological energy conversion the structure and function of this membrane integral multiprotein complex is still poorly understood. Recent insights into the structure of complex I by X-ray crystallography have shown that iron–sulfur cluster N2, the immediate electron donor for ubiquinone, resides about 30 Å above the membrane domain and mutagenesis studies suggested that the active site for the hydrophobic substrate is located next to this redox-center. To trace the path for the hydrophobic tail of ubiquinone when it enters the peripheral arm of complex I, we performed an extensive structure/function analysis of complex I from *Yarrowia lipolytica* monitoring the interaction of site-directed mutants with five ubiquinone derivatives carrying different tails. The catalytic activity of a subset of mutants was strictly dependent on the presence of intact isoprenoid moieties in the tail. Overall a consistent picture emerged suggesting that the tail of ubiquinone enters through a narrow path at the interface between the 49-kDa and PSST subunits. Most notably we identified a set of methionines that seems to form a hydrophobic gate to the active site reminiscent to the M-domains involved in the interaction with hydrophobic targeting sequences with the signal recognition particle of the endoplasmic reticulum. Interestingly, two of the amino acids critical for the interaction with the ubiquinone tail are different in bovine complex I and we could show that one of these exchanges is responsible for the lower sensitivity of *Y. lipolytica* complex I towards the inhibitor rotenone. This article is part of a Special Issue entitled: 17th European Bioenergetics Conference (EBEC 2012).

1. Introduction

NADH:ubiquinone oxidoreductase (complex I, EC 1.6.5.3) is the largest membrane protein complex found in the respiratory chain of many bacteria and mitochondria [1–3]. It plays a central role in oxidative phosphorylation by transferring electrons from NADH to ubiquinone and converting the redox energy of this reaction into an electrochemical proton gradient that drives ATP synthase. In recent years an increasing amount of structural information from X-ray crystallography studies of bacterial [4–6] and mitochondrial [7] complex I has emerged. Nevertheless, complex I still remains the least understood component of the respiratory chain by far. Complex I consists of three functional modules: the N (NADH oxidation) module, the Q (ubiquinone reduction) module and the P (proton pumping) module [8]. Prokaryotic complex I comprises the 14 central subunits with a molecular mass of some 550 kDa representing the minimal form that harbours the bioenergetic core functions [9,10]. The more than 40 subunits of mitochondrial complex I e.g. from *Bos taurus* or from the obligate aerobic yeast *Yarrowia lipolytica* have a molecular mass of almost 1 MDa [11–13]. The membrane arm of L-shaped eukaryotic complex I is embedded in the inner mitochondrial membrane and the peripheral arm protrudes orthogonally into the mitochondrial matrix space ([14]; Fig. 1). The peripheral arm harbours all redox-active cofactors, one FMN molecule and eight canonical iron–sulfur clusters [15] and the active sites for both substrates NADH and ubiquinone.

The ubiquinone-reducing catalytic core resides at the interface of the 49-kDa and PSST subunits [16]. X-ray crystallographic data show that the electron-donating iron–sulfur cluster N2 is located 25–30 Å

Abbreviations: DBQ, n-decyl-ubiquinone; DQA, 2-decyl-4-quinazolinylamine; FMN, flavin-mononucleotide; HAR, hexaammineruthenium(III)-chloride; NADH, nicotinamide adenine dinucleotide (reduced form); Q_1, ubiquinone-1; Q_1S, ubiquinone-1 with saturated side chain; Q_2, ubiquinone-2; Q_2S, ubiquinone-2 with saturated side chain

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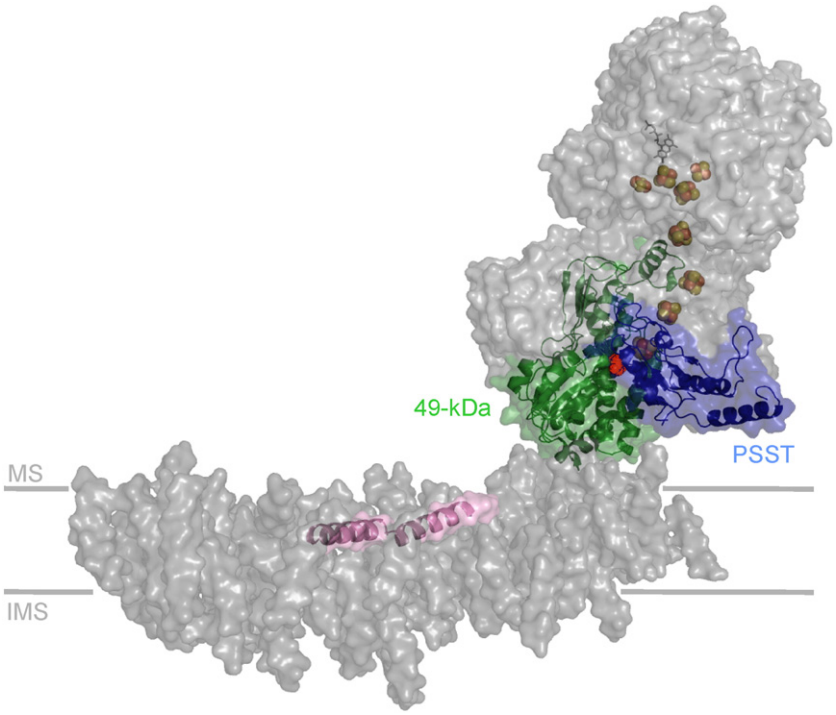

Fig. 1. Position of the ubiquinone reducing site within a structural model of complex I.The overall architecture of the central domains of complex I is illustrated based on X-ray structural data [7]. The PSST (blue) and 49-kDa (green) subunits, forming the ubiquinone binding cavity of complex I, are shown in surface and cartoon representation. Red, residue Y144 of the 49-kDa subunit in space fill representation marking the binding site of the ubiquinone head group; orange/yellow, iron–sulfur clusters in space fill representation; grey sticks, FMN; pink, part of the transmission helix connecting the proximal and distal module of the membrane arm [7]; grey lines, approximate position of the membrane; MS, matrix space; IMS, intermembrane space.

above the membrane plane (Fig. 1; [5,7]) supporting the suggestion that the ubiquinone head group has to move into a deep binding pocket within the peripheral arm to reach its electron donor [17]. Functional characterization of site-directed mutations suggested that the conserved Y144 of the 49-kDa subunit that resides only 6–8 Å away from cluster N2 is a key amino acid residue of the ubiquinone binding site [18,19]: replacing this tyrosine with phenylalanine results in almost complete loss of catalytic activity when n-decyl-ubiquinone (DBQ) is used as a substrate. In contrast, with ubiquinone-1 (Q_1) and ubiquinone-2 (Q_2) that carry short isoprenoid substituents, electron transfer and proton pumping activities are normal, although with a marked increase in apparent K_M values. These data indicate that a hydrogen bond between the phenolic OH-group of the tyrosine and one of the ubiquinone head group carbonyls represents the dominant binding interaction and that at elevated substrate concentrations an isoprenoid tail, but not an aliphatic tail can contribute sufficient affinity to maintain productive substrate binding. Here we further probed this hypothesis and applied mutagenesis based structure/function analysis together with systematically modified substrates synthesized by chemical means, to map the corresponding binding interactions with the first two isoprene units of ubiquinone to trace its tail within the active site of complex I.

2. Materials and methods

2.1. Synthesis of ubiquinone derivatives ubiquinone-1 with saturated side chain (Q_1S) and ubiquinone-2 with saturated side chain (Q_2S)

The synthesis of the substrate-analogues Q_1S and Q_2S starts from the same precursor coenzyme Q_0, which was prepared according to a published procedure in large scale [20]. The alkyl-chains were introduced by a radical Hunsdiecker decarboxylation of the corresponding acid using silver nitrate and peroxydisulfate in an acetonitrile/water mixture, a powerful method to modify the quinone-scaffold [21–23].

For Q_1S, 4-methylvaleric acid (**1**) was coupled to Q_0 under Hunsdiecker decarboxylation conditions. Interestingly, based on the NMR spectrum of the isolated material, only Q_1S was formed as the single desired isomer among three possible isomers. This finding indicated that the Michael-addition of the alkyl radical to the quinone is kinetically much faster compared to the rearrangement of the alkyl radical by hydrogen abstraction to form the other, more thermodynamically stable isomers 1 and 2 (Scheme 1).

1 g (5.4 mmol) Q_0, 0.76 g (6.6 mmol, 1.2 eq.) 4-methylvaleric acid and 0.25 g silver nitrate were dissolved in 20 ml acetonitrile/water 1:1 mixture under argon atmosphere. To this mixture, a solution of 2.8 g (12.2 mmol) ammonium persulfate dissolved in 15 ml water was added dropwise. The mixture was heated to 80 °C for 2 h and stirred at room temperature for 24 h in dark. After extraction of the crude product with ethylacetate and successive washing of the organic phase with saturated $NaHCO_3$ the crude product Q_1S was purified by column chromatography with hexane/ethylacetate 4:1 ($R_f = 0.48$) to give 320 mg product (24% yield).

^{1}H-NMR (300 MHz, $CDCl_3$): δ[ppm] = 3.92 (s, 6 H, 2 × OMe), 2.40–2.34 (t, ^{3} J = 9.0 Hz, 2 H, CH_2), 1.93 (s, 3 H, CH_3), 1.58–1.49 (m, 1 H, CH), 1.21–1.13 (m, 2 H, CH_2CH), 0.88 (s, 3 H, CHCH_3), 0.86 (s, 3 H, CHCH_3).

^{13}C-NMR (75.4 MHz, $CDCl_3$): δ[ppm] = 184.6 ; 184.05 (C = O), 144.3; 144.2; 143.3; 138.4 (C), 28.4 (CH), 37.6; 24.3 (CH_2), 61.1; 61.08; 22.2; 22.2; 11.7 (CH_3).

For Q_2S the same procedure was applied. The carboxylic acid (**4**) needed for the Hunsdiecker decarboxylation is not commercially available and was synthesized in three synthetic steps, starting from commercially available alkyl-bromine (**2**). Kolbe nitrile synthesis [24] formed nitrile (**3**) in good yield, which was subsequently hydrolyzed to afford the desired carboxylic acid (**4**).

Unfortunately, no conversion was observed when carboxylic acid (**4**) was used and only the starting materials were recovered. The synthesis plan was changed and Q_2S was prepared independently by