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Cryo-EM structure of respiratory complex I reveals a link to mitochondrial sulfur metabolism

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ABSTRACT

Mitochondrial complex I is a 1 MDa membrane protein complex with a central role in aerobic energy metabolism. The bioenergetic core functions are executed by 14 central subunits that are conserved from bacteria to man. Despite recent progress in structure determination, our understanding of the function of the ~30 accessory subunits associated with the mitochondrial complex is still limited. We have investigated the structure of complex I from the aerobic yeast *Yarrowia lipolytica* by cryo-electron microscopy. Our density map at 7.9 Å resolution closely matches the 3.6–3.9 Å X-ray structure of the *Yarrowia lipolytica* complex. However, the cryo-EM map indicated an additional subunit on the side of the matrix arm above the membrane surface, pointing away from the membrane arm. The density, which is not present in any previously described complex I structure and occurs in about 20 % of the particles, was identified as the accessory sulfur transferase subunit ST1. The *Yarrowia lipolytica* complex I preparation is active in generating H_2S from the cysteine derivative 3-mercaptopyruvate, catalyzed by ST1. We thus provide evidence for a link between respiratory complex I and mitochondrial sulfur metabolism.

1. Introduction

Mitochondrial NADH:ubiquinone oxidoreductase (respiratory complex I) is a 1 MDa multi-subunit membrane protein complex that has a central role as a proton pump in eukaryotic energy conversion [1,2]. Complex I couples electron transfer from NADH to ubiquinone to translocation of protons across the inner mitochondrial membrane, generating about 40% of the proton motive force that drives ATP synthesis. Complex I has been shown to release toxic oxygen radicals that contribute e.g. to tissue damage in myocardial infarction [3]. Complex I dysfunction is a common cause of mitochondrial disorders and has been implicated in the pathogenesis of neurodegenerative diseases [4,5]. Fourteen central subunits that are conserved from bacteria to man form the catalytic core of complex I. The core subunits, which are sufficient for function, are assigned to three functional modules: the N module for NADH oxidation, the Q module for ubiquinone reduction and the P module for proton pumping [2,6]. The precise roles of the ~30 accessory subunits of eukaryotic complex I are not yet thoroughly characterized [7].

The structure of the core subunits has been determined by X-ray crystallography of bacterial complex I [8–10]. The structure of the mammalian complex was determined by cryo-EM at 5 Å resolution [11] and more recently at 4.2 and 3.9 Å [12,13], and an intermediate-resolution X-ray structure of a bovine complex I fragment has been reported [14]. Together, these structures provide the topology of all mammalian accessory subunits. The 3.6–3.9 Å X-ray structure of mitochondrial complex I from the aerobic yeast *Yarrowia lipolytica* [15] provided insights into the mechanism of redox-linked proton translocation and a regulatory switch between active and deactive forms of the enzyme. However, detailed information on the structure and function of accessory complex I subunits is still limited.

The family of sulfur transferases (E.C. 2.8.1) comprises a group of proteins that catalyze the formation and interconversion of compounds containing sulfane sulfur atoms [16]. Sulfur transferases are present in organisms of all phyla. Although these proteins differ significantly at

Abbreviations: 2D, two-dimensional; 3D, three-dimensional; cryo-EM, cryo-electron microscopy; CTF, contrast transfer function; FSC, Fourier Shell Correlation; MST, 3-mercaptopyruvate:sulfur transferase; MTF, modulation transfer function; SQR, sulfide quinone reductase; TMH, transmembrane helix; TST, thiosulfate:sulfur transferase.

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the sequence level, the tandem domain three-dimensional structure seems to be highly conserved and resembles that of bovine rhodanese, the most studied and best characterized sulfur transferase [17].

Abdrakhmanova et al. [18] showed that a sulfur transferase is associated with *Y. lipolytica* complex I. The recent development of direct electron detectors, improvements in image processing software algorithms and faster computers have made it possible to produce high-resolution maps by cryo-EM [19,20] and to separate particles with different conformations or subunit composition in the same dataset [21,22]. We here report the cryo-EM structure of *Y. lipolytica* complex I. Roughly 20% of the complexes contributing to the map had an additional density on the matrix arm, which was identified as the accessory complex I sulfur transferase subunit ST1. We discuss implications for a link between complex I and mitochondrial sulfur metabolism.

2. Materials & Methods

2.1. Protein purification and functional characterization

Complex I was purified in dodecyl-maltoside by His-tag affinity and size exclusion chromatography [23]. MST activity of complex I was measured using the lead sulfide assay [24,25]. Briefly, the assay mixture containing 100 mM HEPES buffer pH 7.4, 0.4 mM $PbNO_3$, 100 μg/ml BSA, 30 mM DTT and varying amounts of 3-mercaptopyruvate was incubated at 37 °C for 4 min. The reaction was started by addition of the protein sample and formation of PbS was monitored at 390 nm with a Shimadzu UV-2450 spectrophotometer.

2.2. Cloning, protein expression and purification of recombinant ST1

The codon-optimized *st1* gene from *Y. lipolytica* was cloned into the expression vector pET-26b (Novagen, Germany) harboring a C-terminal His_6-tag. The recombinant protein was overexpressed in *E. coli* BL21(DE3) pLysS cells (Novagen, Darmstadt) using liquid broth medium at 37 °C and 150 rpm agitation. Expression of ST1 was induced at OD600 of 0.5–0.6 by adding IPTG to a final concentration of 1 mM. Cells were harvested, resuspended in 50 mM K-phosphate pH 7.5, 200 mM NaCl and broken by several passages through a cell disruptor (model TS, I&L Biosystems, Germany). Insoluble components were removed by centrifugation at 50,000 x g at 4 °C. The supernatant was loaded onto a HisTrap HP column (GE Healthcare; USA) and eluted with a step gradient of 20 mM, 40 mM and 250 mM imidazole. The protein was concentrated by ultrafiltration (Amicon Ultra-4, 10 kDa cutoff; Millipore, Germany) and loaded onto a Superdex 75 10/300 GL gel filtration column (GE Healthcare) equilibrated with 30 mM Tris-HCl pH 7.5, 100 mM NaCl and 5% glycerol. Protein was concentrated to 10 mg/ml and stored at 100 K.

2.3. Cryo-EM data collection

3 μl of a complex I sample at a concentration of 3 mg/ml was applied to freshly glow discharged Quantifoil R2/2 holey carbon grids (Quantifoil Micro Tools, Germany) that had been pretreated in chloroform for 1–2 h. The grids were blotted for 8–10 s at 70% humidity and 10 °C in an FEI Vitrobot plunge-freezer. Cryo-EM images were collected on a FEI Tecnai Polara operating at 300 kV carefully aligned as previously described [26] equipped with a Falcon II direct detector operating in movie mode. Images were recorded manually at a nominal magnification of 59,000× yielding a pixel size at the specimen of 1.77 Å. The camera system recorded 17 frames/s as described [27]. Videos were collected for 1.5 s with a total of 24 frames with a calibrated dose of about 3.5 $e^-/Å^2$ per frame, at defocus values between −1.3 and −4.5 μm.

2.4. Image processing

A set of 2250 micrographs was collected. Whole-image drift correction of each movie was performed using the algorithm developed by [28]. Particles were picked using the semi-automatic procedure of EMAN Boxer [29], and the micrograph-based CTF was determined using CTFFIND3 [30] in the RELION workflow [31]. Where necessary, the contrast transfer function (CTF) was double-checked using the particle-based CTF procedure in EMAN2 [32]. The initial dataset contained 50,314 particle images (224 × 224 pixels) from 2184 selected micrographs. The particles were subjected to two-dimensional reference-free and three-dimensional (2D, 3D) classification in RELION [31] to discard imperfect particles. Visual selection of particle classes with interpretable features resulted in a dataset of 44,936 particle images for the first 3D consensus refinement. A map based on the low-resolution X-ray structure of *Y. lipolytica* complex I [6] was low-pass filtered to 60 Å and used as an initial model for the 3D refinement in RELION. Whole-image drift correction [28] over all the frames was followed by statistical movie processing, using running averages of seven movie frames and a standard deviation of one pixel for the translational alignment. Subsequently the particle polishing procedure implemented in RELION 1.3 was used to account for individual beam-induced particle translations and to calculate a frequency-dependent weight for the contribution of individual movie frames to the reconstructions [33]. The resulting dataset of polished particles was used for further 2D and 3D classification using a soft-edge spherical mask to select the best 3D classes. Several runs of 3D classification with different starting seeds and different number of classes were performed to assess reproducibility and consistency. We then applied first a spherical mask to identify the largest difference between the classes, followed by a soft-edged mask together with finer angular sampling and local angular search. At this stage some 3D classes displayed an extra weak density protruding from the side of the matrix arm. Further particle selection on the basis of extensive 3D classification with a finer angular sampling interval of 3.75° using a soft-edge shaped mask, made with the *relion_mask_create* module of RELION, and a local angular search range of 5° resulted in two smaller datasets, one containing 29,125 particles showing the well-known complex I shape and one of 9485 particles showing a prominent extra density next to the 49-kDa core subunit (NUCM). Further sorting of the particle images recorded close to focus [19] or with high values of the normalized probability distribution [34] did not improve the maps significantly, probably because these procedures work best at high resolution.

The two datasets were subjected to 3D refinement in RELION and the final maps were post-processed for masking, B-factor determination and map sharpening using the post-processing procedure in RELION [35]. After post-processing the resolution was estimated by the 0.143 FSC criterion as 7.9 Å for complex I and 10.4 Å for the complex I – ST1 assembly. A further refinement of the smaller dataset with local angular search was performed in Frealign [36] version 9.11 in mode 1 using the Euler angles, shifts and the soft-edge shaped mask previously obtained with RELION using a global search of 3.75°. This led to an improvement in resolution in the map displaying the extra density from 10.4 to 9.5 Å (Fig. S1).

Before visualization all density maps were corrected for the modulation transfer function (MTF) of the Falcon II direct detector. Maps were sharpened with negative B-factors of 200 $Å^2$ for complex I and 250 $Å^2$ for complex I – ST1.

2.5. Sequence analysis and homology modelling

A homology model of ST1 was built using the PSIPRED Protein Sequence Analysis Workbench [37] (http://bioinf.cs.ucl.ac.uk/) and the HH-Pred toolkit (http://toolkit.tuebingen.mpg.de/hhpred) [38] to find the best match between an available structure and the ST1 sequence. From the best matches sorted by sequence identity (>40%) and coverage SseA, an MST of *E. coli*, (1URH) and the rhodanese-like protein