Multi-protein complex for ubiquinone biosynthesis: biochemical and structural studies

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Ubiquinone, also called coenzyme Q (CoQ or Q), is a lipid molecule that is part of the bacterial plasmatic membrane where it works as an antioxidant and as an electron carrier in the respiratory channel. Ubiquinone is constituted by a redox-active benzoquinone ring substituted by a polypropyl chain of 8 units in *Escherichia coli* (Q8), 9 units in the yeast *Saccharomyces cerevisiae* (Q9) and 10 units in human (Q10) (Fig. 1). Ubiquinone has a crucial role in the functioning of the respiratory channel and in human, primary deficiencies in CoQ as well as mutations affecting the genes of its biosynthesis are associated with diverse severe pathologies (myopathies, renal diseases, cerebellar ataxia, infantile-onset encephalopathy, etc).

![Fig. 1: Structure of ubiquinone and its different redox states](image)

CoQ biosynthesis requires several chemical modifications of its aromatic ring, including one reaction of prenylation, one reaction of decarboxylation, three reactions of hydroxylation, and three reactions of methylation (Fig. 2). In *Escherichia coli*, 9 proteins have been identified as involved in the ubiquinone biosynthetic pathway (UbiA-UbiH, UbiX). Very recently, genetic studies conducted by our collaborators (Fabien Pierrel, CEA-Grenoble and Frédéric Barras, Marseille) allowed the identification of 3 new proteins: VisC (UbiI), YigP (UbiJ) and YqiC (UbiK), all of them involved in the ubiquinone biosynthetic pathway. This pathway is very conserved among species, from prokaryotes to eucaryotes.
Moreover, these proteins seem to form a large multi protein assembly, as this is the case with Coq proteins, the eukaryotic homologues of the bacterial Ubi proteins, involved in Q9 biosynthesis in the yeast *Saccharomyces cerevisiae* (Fig. 3). This high molecular complex is probably membrane-anchored.

Despite the biological importance of ubiquinone in the electron transport system and the involvement of the Ubi proteins in the pathologies described above, the function of each Ubi protein is not entirely known. And a number of uncertainties remain about the identification of the protein(s) responsible for each biosynthetic step, as well as the order of the reactions. Moreover, limited information is available regarding the substrate recognition, specificity, reaction mechanisms as well as the three-dimensional structure of these Ubi enzymes. This is partly because the protein themselves have a tendency to form high molecular weight oligomers and the native substrates of these proteins, the membrane-associated hydrophobic poly-isoprenyl aromatic compounds, are not commercially available and need to be synthetized. Furthermore, they are water-insoluble, and this impedes in vitro enzyme activity assays as well as determination of the crystal structures of the proteins in complex with the substrates. Moreover, obtaining detailed physico-chemical and structural data on purified proteins that are part of a complex in vivo can prove difficult.

We are interested in studying the structure and function of the ensemble of Ubi proteins involved in CoQ biosynthesis in *Escherichia coli*. We aim to precisely determine the biological role of each Ubi protein or enzyme constituting the Ubi multi-protein complex. The in vitro biochemical characterization of Ubi proteins, coupled to the determination of their 3D-structure, will lead to an understanding of their function and mechanism at the molecular level. We also aim to determine their mode of organization in a high weight molecular complex, in terms of supermolecular structure (exact composition of the protein complex, size, stoichiometry and cellular localization) and their hydrophobic anchor to the membrane (collaboration F.Pierrel, UMR5163, Laboratoire Adaptation et Pathogénie des Micro-organismes, UJF Grenoble). Our main goal is to have a deeper knowledge of the E. coli ubiquinone biosynthesis pathway, at the fundamental level. We use a combination of multidisciplinary approaches: biochemistry, molecular biophysics (pressure-perturbation spectroscopy, fluorescence resonance energy transfer, circular dichroïsm, laser flash photolysis, Djemel Hamdane) and structural biology (X-ray crystallography, Ludovic Pecqueur, Béatrice Golinelli).
We have studied UbiI, the enzyme that catalyzes the C5 hydroxylation of 2-octaprenyl phenol (OPP) in the fourth step of ubiquinone biosynthesis. UbiI shares 30% sequence identity with UbiF and UbiH, two other proteins from the ubiquinone biosynthetic pathway that both catalyze reactions of hydroxylation (C1 hydroxylation for UbiH and C6 for UbiF). UbiI, UbiF and UbiH all are Flavin dependent mono-oxygenases. We purified an unstable form of UbiI that was prone to aggregation. After mild proteolysis, we obtained a soluble and truncated form that was lacking its C-terminal domain. This truncated form of UbiI successfully crystallized and we determined its crystal structure (2 Å resolution). It shares high structural similarity with other flavin-dependent monooxygenases, like para-hydroxy benzoate hydroxylase pHBH (Fig. 4). This is the first report of the structure of one Ubi monooxygenase and the first step towards elucidating the active site geometries of these enzymes.

Fig. 4: A- X-ray Three-dimensional structure of an apo and truncated UbiI from E.coli (code PDB 4K22); B- superimposition of UbiI (blue) with PHBH (yellow), code PDB 1PBE (Hajj Chehade, 2014).

We recently started the biochemical and structural study of Coq6 from S.cerevisiae, the eukaryotic homolog of the bacterial UbiI. We have purified it for the first time and showed that it contains FAD as a cofactor (unpublished results). Furthermore, we have shown that this flavoprotein does not take its electron directly from NADPH but from an electron transfer chain consisting of NADPH: ferredoxin reductase (Arh1) and ferredoxin (Yah1) (Fig. 5). We have obtained crystals of an N-terminal MBP-tagged Coq6 that diffracted X-rays to 5 Å resolution and we are currently trying to have crystals of better quality.

Fig. 5: Proposed mechanism of the reduction of Coq6 by Arh1/Yah1

With the aim of a better understanding of Coq6, we generated a model by homology modelling. Molecular docking and molecular dynamics (MD) simulation studies have been performed to explore the putative binding modes of substrate in Coq6. This study allowed us to show the existence of three putative cavities for the substrate access to the active site (Fig. 6).
Fig. 6: Proposed model of the active site of Coq6 (FAD in green), with a putative substrate access tunnel in purple (Caver).

We recently started the biochemical and structural study of UbiX and UbiD, the two enzymes that catalyze the third step of ubiquinone biosynthesis, in which 3-octaprenyl-4-hydroxylbenzoate (OHB) is converted into 2-octaprenylphenol (OPP): this reaction of aromatic decarboxylation is a highly challenging chemical reaction. Despite being isofunctional, UbiX and UbiD share no sequence or structural similarities. We have purified UbiX from E.coli and determined its X-ray structure: we have shown that this is a dodecameric FMN-containing enzyme (Fig. 7) (unpublished results). We are currently investigating the biochemical mechanism of these fascinating flavoenzymes.

Fig. 7: X-ray Three-dimensional structure of UbiX from E.coli (FMN in yellow) (unpublished results)

We also started to study UbiJ and UbiK which are archetypal “new” Ubi proteins. They were not listed among the “Ubi” proteins before our recent studies and they have no predicted enzymatic activity. Previous studies have shown that UbiK from S. typhimurium was shown to promote liposome aggregation in vitro, suggesting a membrane fusogenic activity. UbiK shares 31 % sequence identity with Brick1, a precursor for the assembly of the Wave cytoskeleton complex, a multi-protein complex that promotes actin polymerization. This raises the possibility of UbiK acting as a scaffold protein, playing an important role for the formation, the structuration and the stability of the complex. We have purified UbiJ and UbiK and we have shown that they form a complex (unpublished results). We also obtained crystals of this complex which diffracted X-rays to 7 Å resolution. We are currently trying to have crystals of better quality.

Researchers
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Lab expertise
- Cell culture : bacteria (E. coli)
- Molecular biology and biochemistry: cloning, expression of prokaryotic or eukaryotic recombinant proteins, directed mutagenesis, protein purification
- Enzymology : steady-state and pre-steady state (stopped-flow)
- Optical spectroscopy (UV-visible, fluorescence, CD)
- Characterization of metabolites by mass spectrometry
- Protein Crystallography
- Molecular modelling: protein homology-modelling, docking, molecular dynamics

Collaborations

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- Ecole doctorale ED 387: iViv, Interdisciplinaire du vivant, de l’Université Pierre et Marie Curie Paris VI

References


