anchor peptides and heme *b* composition. Delineation of mechanism of action of these enzymes is highly desirable due to the medical disorders caused by their dysfunctions in eukaryotic organisms. Although several 3D structures of complex II exist, the exact mechanism of action remains unclear. Here we present identification, isolation, purification and characterisation of SQR from an extreme thermophile *Thermus thermophilus,* analysis of which is additionally interesting from the evolutionary perspective and the aspect of protein thermostability. The *Thermus* SQR is constituted by flavoprotein (SdhA), iron–sulfur protein (SdhB) and two membrane anchor proteins (SdhC&D). It has been purified to homogeneity following series of chromatographic steps yielding 8 mg per 100 g of *Thermus* biomass. The EPR analysis revealed unusual influence of succinate on the properties of the [2Fe2S] center and presence of two heme *b* molecules in the protein. The latter places the *Thermus* SQR in the Type A SQOR, crystal structure and detailed characterisation of which are not available to date. The enzyme exists in trimeric form and unexpectedly, it is not stable at temperatures above 70 °C in the purified state as judged by Circular Dichroism analysis. Steady-state kinetic measurements show that the *Thermus* SQR displays standard Michaelis–Menten kinetics at low temperature (30 °C) but exhibits positive cooperativity for succinate oxidation at the optimum temperature of the enzyme (70 °C). To our knowledge, this is the first example of allosterically regulated complex II. To facilitate purification, increase the production yield and enable mutagenesis studies of the *Thermus* complex II, a homologous expression system has been developed. Recombinant form of the enzyme has been produced, purified and its biochemical and biophysical characterisation has also been performed.

doi:10.1016/j.bbabio.2010.04.071

1P.24 Structure of a soluble subcomplex of NADH:ubiquinone oxidoreductase from *Aquifex aeolicus*

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The proton-pumping NADH:ubiquinone oxidoreductase (complex I) catalyzes the transfer of two electrons from NADH to ubiquinone via one FMN and a series of iron–sulfur (Fe–S) clusters. This process is coupled with the translocation of four protons across the membrane. Electron microscopy revealed that complex I is made up of a peripheral and a membrane arm. The peripheral arm contains all known cofactors and the binding site for NADH. We have obtained a subcomplex consisting of subunits NuoE and NuoF by heterologous expression of the corresponding genes from *A. aeolicus* in *E. coli*. This subcomplex comprises the NADH- and FMN-binding sites and harbours two Fe–S clusters. The NuoEF subcomplex was purified to homogeneity by chromatographic methods. The cofactors of the preparation were characterized by EPR- and UV/vis-spectroscopy [1]. The structure of the subcomplex was determined at 1.9 Å resolution. To define the substrate–protein interactions the structure of the subcomplex with nucleotides was examined.

Reference

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doi:10.1016/j.bbabio.2010.04.072

1P.25 The study of cbb_3-type oxidase

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Heme-copper superfamily are terminal respiratory oxidases in mitochondria and in many bacteria, coupling the oxygen reduction to water in order to generate proton-motive force across the membrane. The most established heme-copper oxidases are aa_3-type oxidases followed by the second abundant cbb_3-type oxidases. However, the sequence homology between aa_3- and cbb_3-type oxidases is low and they are highly different within the heme-copper superfamily. aa_3-type oxidases utilize two proton uptake pathways known as K-pathway and D-pathways for both catalytic and pumped protons. Whereas, the pattern of conserved polar residues supports the one proton uptake pathway in cbb_3-type oxidases. cbb_3-type oxidases express under low oxygen tension and have a lower K_M for oxygen than aa_3-type oxidases. Moreover, cbb_3-type oxidases are the closest to the bacterial nitric oxide reductases (NOR), which reduce NO to N_2O. This reaction is not electrogenic and do not pump protons or generate proton-motive force. In this study, we investigate the molecular details of the reasons for the differences between the subfamilies of heme-copper oxidases by focusing on cbb_3-type oxidases.

doi:10.1016/j.bbabio.2010.04.073

1P.26 Kinetic and structural analysis of succinate:ubiquinone oxidoreductase (complex II) inhibition by thiapronil

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Mitochondrial succinate:ubiquinone oxidoreductase (complex II, SQR, EC 1.3.5.1) catalyzes the oxidation of succinate to fumarate as part of the tricarboxylic acid cycle. With ubiquinone (Q) as its terminal electron acceptor the SQR is also an entry point for electrons into the respiratory chain. The catalytic core of complex II consists of a flavoprotein (FP) with covalently bound FAD and an iron–sulfur protein (IP) carrying three Fe–S clusters. These two subunits are anchored to the inner membrane by two small transmembrane subunits [1,2]. The precise structural requirements for ubiquinone binding and the mechanistic details of its reduction remain to be elucidated. X-ray crystallographic studies of Q-site inhibitors bound to SQR have indicated that amino acid residues of the IP and of the transmembrane subunits constitute the putative Q-site [3,4]. Under these circumstances, chemically diverse specific inhibitors may be valuable tools for functional and structural studies of complex II. Evidence is presented here that the cyanoketone insecticide thiapronil [3-(2-chlorophenyl)-3-oxo-2-(4-phenyl-1,3-thiazol-2-yl)propanenitrile] blocks quinone reduction by purified housefly (*Musca domestica*) SQR at low nanomolar concentrations. Non-competitive inhibition is observed with respect to the electron acceptor decylubiquinone. However, Yonetani–Theorell analysis indicates that the established Q site inhibitor atpenin A5 and thiapronil bind in a mutually exclusive fashion. The structure of thiapronil bound to the chicken complex II has been solved at 2.2 Å resolution. A number of interactions contribute to the high binding affinity of thiapronil. Two H-bonds are formed between the carbonyl oxygen and the residues TyrD58 (transmembrane subunit D) and TrpB173 (iron–sulfur protein). The 3.9 Å distance between TrpB173 and the thiazole sulfur