PROJECT SUMMARY

The sulfide oxidase flavocytochrome c (Fcc) and sulfide quinone oxidoreductase (Sqr) enzymes are ancient flavoproteins which play a basic role in the regulation of sulfide content. Based on their activity these enzymes are involved in numerous physiological and pathophysiological processes in a wide range of organisms. Six different types of the sulfide quinone oxidoreductase proteins are known which possess a common basic structure; however, there are significant differences among them in the active center and in other parts of the protein. The aim of this Ph.D. project is the investigation of the structural properties and catalytic mechanism of a model enzyme belonging to a poorly characterized group of Sqr proteins, mainly via the usage of bioinformatic tools. With these studies we would like to get an insight into the working mechanism of the enzyme at a molecular level and into the role of essential structural elements responsible for protein function.

BACKGROUND OF THE STUDY

The most reduced sulfur form, sulfide, is produced by abiotic and biotic ways, too. Despite its toxicity sulfide is involved in a variety of important physiological and pathophysiological processes in the organisms. It can perform as electron donor, intracellular signal molecule or neurotransmitter in the case of different cells and tissues. The flavocytochrome c (Fcc) and sulfide quinone oxidoreductase (Sqr) enzymes are ancient flavoproteins, members of the disulfide oxidoreductase enzyme family. They catalyse the oxidation of sulfide and are widely present in different domains of life. These enzymes play a basic role in the energy metabolism of numerous microorganisms, in the regulation of cellular sulfide concentration in eukaryotic cells and in the protection against toxic sulfide. The membrane bound sulfide quinone oxidoreductases transfer electrons from sulfide directly into the membrane quinone pool which are utilized in the energy conserving processes. Six different types of the sulfide quinone oxidoreductase proteins have been evolved through their evolution. The different types of Sqr proteins possess a common basic structure; however, there are significant differences in their primary structure. Therefore, Sqr enzymes may differ in the structure of their active centre, catalytic mechanism and enzymatic function. Many microorganisms, like the photosynthetic purple sulfur bacterium Thiocapsa roseopersicina, have more than one different types of Sqr enzymes. The specific functions of the different Sqr proteins in the metabolism of a certain cell have not been identified.

RELEVANT RESEARCH IN THE HOST LABORATORY

Our research group intensively studies the structure, catalytic mechanism and metabolic function of sulfide oxidase enzymes. Our model organism is the Thiocapsa roseopersicina which has a versatile sulfur metabolism. Genes
of a flavocytochrome c enzyme (Fcc) and two sulfide quinone oxidoreductase type proteins (SqrD and SqrF) were identified in the genome sequence. Phylogenetic and comparative sequence analysis of these proteins revealed that SqrD and SqrF belong to partially characterized groups of the Sqr type enzymes (type IV and VI, respectively). For the identification of the function of these proteins fcc and sqrF T. roseopersicina strains were created and phenotypically analysed. Furthermore, the effect of sulfide on the expression of the identified genes was studied by qRT-PCR. These analyses revealed that the studied proteins have a role in the oxidative sulfur metabolism of cells at different sulfide concentration conditions. The methods of recombinant homologue expression and purification of the soluble Fcc and the membrane bound SqrF enzymes and their mutant variants were developed in our laboratory. The pure and highly concentrated protein samples are suitable for the experimental analysis of structural features, kinetic properties and catalytic mechanism of the studied sulfide oxidase enzymes. In these studies we use various absorbance spectroscopy and fluorescence spectroscopy methods, protein chromatography and enzyme activity measurements. Based on these experiments we have determined the kinetic parameters of the studied enzymes which revealed that the affinity of Fcc and SqrF to sulfide is considerably different in good correlation with the expression and sulfide metabolism studies. We have detected SqrF homocomplexes and investigated the effect of the quaternary structure on the enzyme activity of the protein.

SPECIFIC AIMS

Our recent results have demonstrated that the SqrF enzyme belonging to the group VI of Sqr type proteins differs from the well characterized sulfide quinone oxidoreductases in some conserved sequence motives and kinetic properties. Based on these previous results it is assumed that the catalytic process of type VI Sqr enzymes is not exactly the same as the known models of the catalytic mechanism of sulfide oxidation in type I and V Sqr enzymes. The primary aim of this Ph.D. project is the investigation of the structural properties and catalytic mechanism of SqrF as a model enzyme of a poorly characterized group (type VI) of sulfide quinone oxidoreductase proteins mainly via bioinformatic approach. In the frame of the project our goals are building the 3D structural model of the SqrF protein and studying the supposed protein-protein interactions between SqrF monomers, the cofactor and substrate binding and the catalytic process of the enzyme, basically by the usage of structural and molecular dynamics bioinformatic tools. This is planned in order to identify the structural elements and amino acids which play a role in these protein functions. The verification of the role of the sequence elements identified by in silico protein analysis will be carried out by preparation and characterization of the corresponding mutant SqrF proteins. With these studies we would like to get an insight into the working mechanism of the enzyme at molecular level and into the role of essential structural elements responsible for protein function. We would like to identify the structural background of the observed special catalytic properties of the type VI sulfide quinone oxidoreductases. In addition, the in silico analysis will be extended for the investigation of the structural and catalytic properties of Sqr proteins belonging to other groups of the enzyme family.

MATERIAL AND METHODS

The identification and characterization of the structure of sulfide quinone oxidoreductase enzymes will be carried out by the usage of bioinformatics methods and tools. Principally molecular mechanical methods will be applied, such as homology modelling and molecular dynamics studies. If necessary higher level calculations (QM/MM) will also be performed. For verification of the in silico protein structural results recombinant T. roseopersicina strains expressing mutant SqrF proteins will be constructed by molecular cloning methods. The enzyme variants will be purified by affinity
chromatography and characterized by different protein chromatography and spectroscopy methods. The kinetic properties will be analysed by activity measurement experiments.

SUGGESTED READINGS


SNAPSHOTS FROM THE HOST LABORATORY

Significant publications


Representative recent research grants

“Transmembrane electron transfer as background for the biological activity of cytochrome b561 proteins” (OTKA, 2013-2017)

Some of the latest students in the laboratory

Duzs Á, Ph.D., 2011-recent; “Characterization of oxidative sulfur metabolism and sulfide oxidase enzymes in the purple sulfur bacterium Thiocapsa roseopersicina.”
Dobrotka P, B.Sc., 2011-2013; “Purification and characterization of a sulfide quinone oxidoreductase type enzyme from Thiocapsa roseopersicina.”
Kiss E, M.Sc., 2012-2014; “Investigation of kinetic and structural properties of the flavocytochrom c enzyme in a phototroph purple sulfur bacterium.”
Németh B, M.SC., 2013-2015; “Expression, purification and biochemical characterization of the sulfide quinone oxidoreductase 2 enzyme of Thiocapsa roseopersicina.”