RESEARCH INTERESTS

Our current research includes several interdisciplinary topics that originated from our long-lasting interest in multienzyme membrane complexes involved in energy transduction during photosynthesis and respiration:

- Molecular mechanisms of electron and proton transfer in biological systems;
- The structure and function of membrane-bound multienzyme complexes;
- Biophysical chemistry of protein-cofactor and protein-protein interaction;
- Biochemical and biophysical properties of photosynthetic reaction centers;
- Bioengineering of light-induced hydrogen production;
- Molecular mechanisms of quinone processing sites;
- Mathematical modeling and analysis of electron transfer and coupled processes in biological systems.

SKETCH OF SOME RESEARCH PLANS

Molecular mechanisms of the cytochrome bc_1 complex family

The cytochrome bc_1 complex (Complex III) occupies a central position in all the main energy transducing systems of mitochondria, bacteria, algae and chloroplasts (the related cytochrome b_6f complex). Enzymes of the bc_1 complex family are directly responsible for approximately a third of the energy transduction of the biosphere. The bc_1 complex is also a major player in cellular aging, being responsible for generation of a substantial fraction of reactive oxygen species that lead to DNA and protein damage. Thus, understanding the molecular mechanisms of the bc_1 complex is of major importance for medical and basic research.

This proposal focuses on the molecular mechanisms of bc_1 turnover, utilizing the complex in purple bacteria. This system has several significant advantages for mechanistic studies. (1) It can be activated by short flashes via the photosynthetic reaction center (RC), allowing time resolution of kinetic events ($\geq 1\mu s$). (2) The subunit composition of the prokaryotic bc_1 complex is much simpler than that of eukaryotes. (3) Methods of genetic engineering are well developed. (4) Histidine-tagged variants of bc_1 complex are now in current use, further facilitating the biochemical and mutagenesis aspect of this work.

The proposed research combines the use of kinetic absorbance spectroscopy, multichannel optical spectroscopy, infrared spectroscopy (FTIR, ATR-FTIR and step-scan FTIR), direct electric potential measurements, with the selective use of mutants in a histidine-tagged background. Specific aims include: (1) identification of principle components of the proton conducting pathways; (2) characterization of molecular mechanisms of coupling between electron transfer and protolytic reactions of the ubiquinones in the bc_1 complex; (3) characterization of the intraprotein electron transfer between b_L and b_H by the field-induced perturbation method; (4) determination of the role of the monomer-monomer electron transfer in the function of the bc_1 complex; (5) characterization of factors controlling superoxide production in the bc_1 complex.

Energy-transducing supercomplexes of respiration and photosynthesis

Membrane proteins are responsible for many key cell functions, such as energy transduction, signal reception, solute transport and others. The energy transducing membrane complexes are frequently organized into larger structural and functional units. In case of photosynthesis they form specialized photosynthetic units, which couple together the capture of light energy, oxidation and reduction of specific substrates and formation of a transmembrane proton gradient. Respiratory enzymes are also frequently organized into supercomplexes, sometimes called “respirasomes”.

Purple bacteria are metabolically versatile and can develop both respiratory and photosynthetic electron-transport chains, depending on the conditions of growth. They provide the ideal system for studying the role of different factors in organization of supercomplexes. The characterization of such energy transducing macromolecular nanomachines is of great significance for the general understanding of principles of energy transduction during respiration and photosynthesis.

In recent years many different scanning and imaging techniques have been developed to study biological structures with (sub)nanometer resolution without the use of fixative treatments. A central role is now played by atomic force microscopy (AFM). AFM has an outstanding signal-to-noise ratio and allows one to determine

subnanometer details of the topography of individual membrane proteins or their complexes under native conditions in aqueous environment, as well as of two-dimensional crystals of purified membrane proteins.

Using combinations of genetic engineering, protein biochemistry, spectroscopic analysis, atomic force microscopy, electron crystallography, capillary hydrodynamic fractionation, dynamic light scattering and 2D native electrophoresis we will study the structure, assembly and function of different multi-component membrane protein complexes *in situ* and in the detergent-solubilized states. Specific aims include: (1) determination of the structure and dynamics of *individual* membrane proteins embedded in native membranes; (2) macromolecular *complexes* and their dynamic formation under different conditions in native membranes; (3) direct visualization of conformational changes of membrane proteins in 2D crystals; (4) intramolecular and intermolecular forces controlling formation of multienzyme membrane complexes in native membranes; (5) functional characterization of different detergent-solubilized supercomplexes trapped in polyacrylamide gel; (6) role of chemical modification of proteins in lateral distribution of membrane complexes; (7) role of lipid-protein and protein-protein interaction in formation of supercomplexes; (8) The functional necessity of the supercomplexes for efficient electron transport and energy transduction.

The biochemistry, biophysics, molecular biology and evolution of quinone processing sites

The importance of this study is stressed by multiple indications that coenzyme Q_{10} can be an essential component of anti-aging strategies due to its natural antioxidant properties that can protect the body from free radicals. As a result, it can prevent mitochondria damage, which can be responsible for multiple neurodegenerative processes leading to Alzheimer and Parkinson diseases. There are also some indications that Q_{10} can be important in fighting cardiovascular disease. Thus, understanding the molecular mechanisms of ubiquinone interaction with lipids and proteins is essential for understanding and fighting the aging process.

This proposal focuses on the molecular mechanisms of interaction of quinones with respective binding sites in different molecular complexes of respiration and photosynthesis, such as bc_1 complex, succinate dehydrogenase, and others, for which detailed molecular structures have been determined in recent years. The proposed research combines different experimental methodologies (infrared spectroscopy (FTIR, ATR-FTIR, step scan FTIR), multichannel optical spectroscopy, kinetics absorbance spectroscopy, EPR, NMR, direct electric potential measurements, dynamic light scattering, microcalometry, different electrochemical methods), with the selective use of mutants of individual complexes in a histidine-tagged background. To test current hypotheses we will use detergent solubilized protein complexes, liposomes and native membranes. Different natural and artificial quinones having different tail length will be studied to understand the role of the quinone's head and tail structures in these processes.

Specific aims include: (1) determination of main factors controlling the quinone recognition by the protein; (2) modulation of the quinone properties by the protein; (3) mechanisms of coupling of different membrane complexes with quinone pool; (4) mechanisms of semiquinone stabilization in the protein; (5) factors controlling the differentiation between quinones and phospholipids; (6) Modulation of quinone properties by a protein (shift E_ms, pKs, stability of semiquinone); (7) Coupling of quinones with protons and role of protein in this process; (8) factors controlling the adapting different types of quinones in different types of organisms; (9) General comparative characterization of different types of quinone binding sites; (10) general properties of quinone binding sites; (11) molecular modeling of quinone processing sites; (12) comparative analysis and evolution of quinone processing sites.

Bioengineering of reaction centers

The initial events of photosynthesis occur in a membrane-bound pigment-protein complex called the reaction center (RC). Reaction centers of photosynthesis in plants, algae and a variety of bacterial species are integral membrane proteins, which are responsible for the conversion of light energy into chemical free energy. Significant progress has been made in recent years in biochemical and biophysical characterization of reaction centers (RCs). Several RCs from different sources, including Photosystem II and Photosystem I, have been crystallized and their structures have been determined at atomic resolution. As a result, cofactors of the RC proteins were localized and the role of the individual amino acid residues in cofactor binding has been identified.

It was found that photosynthetic reaction centers differ significantly in their composition and nature of the terminal donors and acceptors. In spite of these differences, all known types of photosynthetic RCs show

significant similarity in the arrangement of their cofactors involved in the light-induced transmembrane charge separation. In all studied cases the general outline of reaction centers includes two symmetrically arranged branches of six porphyrin derivatives and two quinones. With this general outline, RCs in different organisms were able to accommodate (by changing the type of quinone and porphyrin cofactors, or by modifying specific protein-cofactor interactions) different donors and acceptors.

In spite of significant biochemical, biophysical and molecular biological studies on PS II, the understanding of the molecular mechanisms governing its function on the acceptor side is still very crude. The reasons for this include: (i) complex polypeptide composition of the oxygen-evolving PSII core complexes, (ii) the presence of the strongly-absorbing intrinsic chlorophyll-binding proteins, complicating the application of optical methods, (iii) relative instability of isolated complexes, (iv) the internal heterogeneity of oxygen-evolving PSII core complexes, and (v) significant interference of measured signals originating from donor and acceptor sides. To overcome these problems it is proposed to employ a new system - a hybrid membrane protein which combines the essential part of the protein on the acceptor side of PS II and the donor side of bacterial RC. This will allow us to focus on the outstanding questions concerning the molecular mechanisms of the acceptor quinone complex of PS II, utilizing the advantages of bacterial RCs - their stability and suitability for optical, structural and molecular biology studies. Specific aims of this project include: (1) Creation of different mutants closely simulating the acceptor side of PSII; (2) Quantitative characterization of the kinetics and thermodynamics of electron transfer and coupled reactions in acceptor plastoquinones in such RCs (identification of amino acid residues involved in the binding of PS II plastoquinone acceptors; (3) the localization and characterization of protonogenic and electrogenic steps in chimera turnover; (4) identification of the amino acid residues involved in the formation of proton transfer chains; (5) characterization of fast electrochromic bandshifts induced by the acceptor plastosemiquinones; (6) interaction between quinone binding sites in PS II); (7) Clarification of the role of bicarbonate in regulating electron transport and proton delivery on the acceptor side of PSII; (8) Analysis of species-specific herbicide binding to the acceptor side of PSII; (9) Analysis of the mechanisms of reductive photodamage in PSII-like bacterial RC and role of cytochrome b559 therein.

Light-induced hydrogen production

Current energy production and use is the main source of environmental pollution and global climate change. Moreover, fossil fuel reserves are limited and the cost of traditional energy supplies will continue to increase. Thus, alternative energy sources have to be developed that complement or replace current usage of the fossil fuel reserves. These energy sources should be renewable, ecologically clean, and reasonably cheap, and use the sun as primary source of energy. Hydrogen is an ecologically clean form of energy. Its usage in addition to the traditional fossil fuel can significantly reduce emission of carbon to the atmosphere.

Energy transduction in biomembranes during respiration and photosynthesis is an example of “hydrogen energetics” during which main reaction is either oxidation of NAD(P)H (one can view NAD(P)H as kinetically stable equivalent of hydrogen molecule) by oxygen to produce energy as in respiration, or usage of light energy to oxidize water to produce NAD(P)H, as in oxygenic photosynthesis.

This proposal focuses on the development and usage of different photosynthetic systems that can be used for hydrogen production. In particular we address the problem of the reconstituting water-splitting light-driven system for hydrogen production from individual protein complexes and enzymes. This system will employ individual components involved in the photoinduced hydrogen production, namely Photosystem II, Photosystem I from thermophilic cyanobacteria and oxygen-tolerant hydrogenase, immobilized on an electrode surface and connected by natural or artificial electron donor-acceptor systems.

First steps to the creation of a virtual mitochondrion and virtual chloroplast. Quantitative analysis of electron transport in multienzyme complexes.

In some metabolic pathways, several enzymes are organized into multienzyme complexes, increasing the overall efficiency of the pathway. The electron transport chains of energy-transducing biomembranes consist of relatively small number of interacting multienzyme complexes, in which the redox work is coupled to the generation of a proton gradient needed for ATP synthesis. Many of these complexes have been crystallized recently and their detailed structures made available. While the qualitative understanding of many processes in such complexes has been achieved as the result of intensive biochemical, biophysical and molecular biology studies, their quantitative description is only beginning to emerge.

The principal difficulties of quantitative description of electron transport in such complexes arise from the large number of interacting cofactors and electron carriers, non-linear interactions between some electron carriers, coupling of electron transport with generation of a proton gradient, from internal heterogeneity of the system and necessity of parallel consideration of different interacting processes at molecular, intermolecular and membrane levels.

This proposal focuses on the mathematical modeling of the molecular mechanisms of turnover of individual multienzyme complexes in energy transducing membranes, such as photosynthetic reaction centers, cytochrome bc_1 complex, etc. Mathematical modeling will be used to describe kinetic and thermodynamic data obtained by optical differential spectroscopy, multichannel spectroscopy, and electrometric methods. The principal objectives of this proposal are building and testing mathematical models of electron transport and coupled processes of individual multienzyme complexes of energy-transducing membranes. Such mathematical modeling will: (1) provide the framework for testing current views and hypotheses about function of the particular complex; (2) allow prediction of the behavior of a complex under conditions (or for values of parameters) for which experimental data are currently absent, and in mutant strains; (3) provide convenient tools for organizing large experimental data sets describing kinetic and thermodynamic behavior of a complex; (4) provide a concise set of parameters (rate and equilibrium constants, thermodynamic parameters, extinction coefficients, etc.) characterizing energy transduction in a complex; (5) allow extension of the modeling exercise to quantitatively evaluate the effects of interaction of the particular complex with other components of electron-transport chain; (6) allow extension of the modeling environment to include development of software advances through which kinetic modeling can be linked to a wider set of databases.

TEACHING INTERESTS

My educational background includes Biophysics (Biophysical Chemistry) and Biology (MS), Biophysics (Biophysical Chemistry, PhD) and mathematics (Applied mathematics, MS).

I have extensive teaching experience. I have taught bioenergetics, photosynthesis, stochastic processes in biochemistry and biophysics, mathematical statistics and others. I had also organized a lecture-discussion seminar for undergraduate students in biophysics and bioenergetics. Many students who obtained training through this seminar became internationally recognized scientists in biochemistry and biophysics (Cherepanov D.N., Mulkidjanian A.YA., Pottosin I.I., Sled' V.D., Zherdev A.V. among others).

At the University of Illinois I was involved in teaching Biophysics 354 (Biological energy conversion) and Biophysics 332 (Photosynthesis).

I have (co)authored 3 books entitled "Electron Transfer in Biological Systems"; "Function of Quinones in Bacterial Photosynthesis", and "Thermodynamics of Biological Processes" (all in Russian).

As a result of my work in multidisciplinary environment, and my previous experience with teaching and research, I would feel comfortable teaching in the following areas:

- Biophysical Chemistry/Physical Biochemistry/General and Molecular Biophysics
- Biochemistry
- Membrane biophysics
- Plant physiology
- Bioinformatics/Chemometrics/ Multienzyme systems/Regulation of Biological Processes
- Kinetics and Thermodynamics of Biological Processes