

Introduction

Extremophiles: Fascinating Organisms with Surprising Capabilities

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Our knowledge of membrane structure and functions as presented in textbooks is usually based on results of research on organisms living in environments characterized by moderate conditions regarding temperature, pH, salinity or oxygen pressure, etc. At least these conditions are routinely considered as "normal," neglecting the fact that presumably life originated under environmental circumstances far apart from those. Actually, a large number of microorganisms have been discovered within the past decade which apparently are thriving in more or less bizarre habitats as deep sea black smokers, alkaline lakes, hot sulfurous springs, natural or artificial salt lakes, and extremely acidic environments like puddles of solutions seeping from ores or mine-refuse piles.

These so-called "extremophiles" are a great challenge to scientists from multiple disciplines. A few aspects may be highlighted, such as (1) there are new and unusual metabolic pathways and enzymes to be discovered, (2) the structural principles of proteins exhibiting extreme thermostability and assuming their native state only at temperatures close to the boiling point of water, (3) the use of either whole organisms or purified enzymes as catalysts in biotechnological processes necessitating extreme conditions, (4) the study of evolutionary pathways in the development and differentiation of catalytic protein domains, and finally (5) development of new experimental approaches for culturing, sampling, and preparatory procedures often needed for the study of "extremophiles"

or their constituents. Thus, the field offers an ideal set of problems for multidisciplinary efforts calling for the cooperation of microbiologists, geochemists, enzymologists, biophysicists, molecular biologists, protein chemists, and biotechnology engineers.

Interestingly, the majority of species characterized as "extremophiles" belongs to a distinct branch of the universal tree of life which has become indisputable as the "third kingdom" of the so-called archaeobacteria (archaea). These are strikingly unique in many details of their molecular genetics, membrane structure, and capabilities to utilize light or chemolithotrophic processes for energy conservation.

In addition, research on archaeobacteria is of particular interest for an understanding of early stages of the evolution of organisms, the evolution of survival strategies, and structure-function relations of specific proteins especially of membrane components involved in energy transduction.

Though there is no serious argument against the statement that the chemiosmotic principles for energy conservation and solute transport are verified also for archaeobacteria and extremophiles, they have to cope with special problems as in the case of alkaliphilic bacteria, apparently exposed to an "inversed proton gradient", or with thermoacidophilic organisms whose membranes have to withstand excessive proton gradients at high temperatures.

This volume is intended to highlight certain key problems and their solutions from the wide field of archaeobacteria and extremophilic organisms. Certainly, a complete coverage of the entire range is impossible and would necessitate a series of largely diver-

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sified issues. As an "appetizer" to those who want to join the dialogue, and as a short summary to the state of the art in archaeobacterial bioenergetics, a number of topics have been selected which provide new insights, on the one hand ready for the textbook, on the other challenging controversial further discussion.

The remarkable stability of the membranes of many thermophilic and thermoacidophilic archaeobacteria allowing them to withstand high temperatures is partially based on the exceptional structure of the cell surface layer. The crystalline-like structures of protein assemblies are reminiscent of the esthetic appeal of virus envelope symmetries. Besides their stabilizing effect by strong interactions within the oligomers and their tight interdigitation with the plasma membrane, a novel aspect is the existence of a quasi-periplasmic space under "dome-like" compartments. Previously it was an accepted idea that archaeobacteria would not possess such a periplasmic space at all. Another interesting feature is the anchoring of these regular surface structures by pillar-like protrusions into the plasma membrane. Due to their fixed localization within the crystalline-like network, these may represent a regular array of pillars within the lipid layer of the membrane, significantly hindering or eventually even arresting lateral diffusion of larger protein assemblies such as respiratory electron transport complexes.

The unique tetraether and diether lipids of archaeobacteria have now been classified and analyzed in great detail. The structural data indicate a close similarity within species of the same genus. Eighty to ninety percent of the membrane lipids in sulfur-metabolizing archaea are of the tetraether type, while diether core lipids are typical for many methanogens. As an important feature, these lipids bear glycosidic oligosaccharides facing the outside of the membrane and form a lipid monolayer as the basal membrane matrix. Their remarkable stability under extreme environmental conditions may offer interesting applications to biotechnology. They also provide an excellent material to generate liposomes with unique properties regarding thermostability and tightness against solute leakage.

As an organelle-like subcellular membrane, the gas vesicles of halobacteria are a device to regulate the buoyant density of the cells. The biosynthesis of this membrane has been shown to involve up to 14 genes arranged in three transcription units. This complexity is surprising in view of the fact that only one major protein constituent is found in the intact vesicle

membrane. The approach reviewed in this volume may provide a general basis for the synthesis of such vacuoles as also found in cyanobacteria, for example.

As to the processes of energy conservation, methanogenic bacteria seem to display the closest relation to archaic conditions presumably prevailing during early evolution. They thrive in strictly anaerobic habitats using a number of reductive metabolic pathways involving simple substrates as hydrogen, CO₂, acetate, methanol, or methylamines. They deserve scientific study not only for their importance in biotechnological applications but also as a major factor influencing global atmospheric composition. During investigation of the mechanism of methanogenesis, a number of new and unique cofactors were discovered, enabling these organisms to generate an ion-motive force across their plasma membrane based on proton- or sodium-ion gradients; the latter serve as a source of energy for the synthesis of ATP, for reversed electron transport, or for solute uptake.

Phototrophic archaeobacteria transform light energy by several rhodopsins, whereas no chlorophyll-containing reaction centers have been detected as yet within this kingdom of organisms. The mechanisms of retinal-linked energy transformation have been dealt with in a preceding issue of "mini-reviews" (Vol. 24, No. 2, April 1992). Therefore, this volume focuses only on a comparison of sequence homologies between several proton pumps of the rhodopsin type, stressing on the overall process of oxidative or photophosphorylation in halobacteria. In confirmation and extension of data obtained with *Sulfolobus*, the previously unexpected similarity of archaeobacterial ATP-synthases and eukaryotic vacuolar ATPases became an important issue. A recent volume of this journal (Vol. 24, No. 3, 1992) focused on various types of ATPases. Nevertheless, it should be emphasized that archaeobacterial ATP-synthase functionally and genetically is of chimeric nature, combining constituents of both F₀F₁-type and V-type ATPases. A comparative discussion of functional implications is published elsewhere (Schäfer and Meyring-Vos, 1992a,b).

Aerobic archaeobacteria are found among halobacteria and the thermoacidophilic sulfur-metabolizing archaea. Our knowledge of their electron transport systems is still fragmentary despite the finding that, for example, in *Sulfolobus* a simplified respiratory chain exists containing the first described aa₃-type terminal oxidase, acting as a quinol-oxidase (Anemüller and Schäfer, 1989; 1990). Although this

enzyme and constituent polypeptides from another archaeobacterial *aa₃* cytochrome have been sequenced (Lübben *et al.*, 1992; Denda *et al.*, 1991), a review of the complete respiratory system in archaea should be postponed to a future date because of the present state of knowledge.

Many bacteria live at extremely acidic or alkaline conditions. The principal mechanisms enabling acidophilic bacteria to maintain moderate internal pH have been reviewed (Bakker, 1990; Matin, 1990). As long as their proton-extruding apparatus is working, they will have no problem in generating ATP via a proton-coupled ATP-synthase. In the case of alkaliphilic bacteria, the question arises which coupling ion is replacing the proton, if any. This question is reviewed considering the use of membrane potential instead of a pH gradient, as well as the possibility of an intermediate exchange of sodium versus hydrogen ions. Also, some speculative ideas are under dispute, locating the chemiosmotic events in microenvironments not accessible to the bulk aqueous phase.

Organotrophic bacteria must accumulate solutes as the primary energy source from their environment. Under extreme conditions such as high temperature or acidic (respectively, alkaline) pH, the function of transport systems as known from mesophilic organisms may be impaired. Obviously, adaptations to such tasks enable these organisms to survive in unusual habitats; these adaptations can now be investigated on

a genetic level. Regarding archaeobacteria and other extreme thermophiles, the recent development of reconstitution techniques, using archaeobacterial membrane-spanning lipids, is of special interest. It promises future approaches to allow a rigorous investigation of various isolated carrier systems from extremophiles in their natural environment.

Though many other focal points of modern research on extremophiles may be raised which cannot be touched in this series of mini-reviews, the present collection may serve as a challenge for further research and discussion of membrane bioenergetics on the level of function, structure, genetics, and application of membrane proteins from organisms inhabiting unusual biotopes.

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