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The Evolution of Energy-Transducing Systems. Studies with an Extremely Halophilic Archaebacterium.

Semiannual Progress Report, February 1991 - July 1991

NASA Cooperative Agreement number: NCC 2-578[1)]

Principal Investigator: Helga Stan-Lotter

Summary:

The halobacterial ATPase was labeled with ^{14}C-dicyclohexylcarbodiimide and subunit II of the enzyme was prepared by electroelution. Subunit II was cleaved by several chemical and enzymatic procedures for further preparation of peptides.
Immunoreactions (Western blotting) of halobacterial membranes were performed with an antiserum against subunit A of the vacuolar ATPase from *Neurospora crassa*. . A 85 K band (subunit I) from the membranes of *H. saccharovorum* and from two halobacterial isolates, which were isolated from Permian salt sediments, reacted strongly with the antiserum. The ATPase from the latter isolates resembled the ATPase from *H. saccharovorum*, but had a higher content of acidic amino acids. If it can be verified that the age of the bacterial isolates is in the same range as when deposition of salt occurred, an extremely interesting system for the study of evolutionary questions would be available, since the salt-embedded bacteria presumably did not undergo mutational and selectional events.

[1]The NASA Technical Officer for this grant is Dr. L.I.Hochstein, NASA Ames Research Center, Moffett Field, CA 94035

[2]Abbreviations: DCCD, dicyclohexylcarbodiimide; NEM, N-ethylmaleimide.

Progress report

1. The ATPase from *Halobacterium saccharovorum* is inhibited by DCCD[2)], as are F-type ATPases. The halobacterial subunit which incorporates the bulk of ^{14}C-DCCD is subunit II, a 60 000 dalton protein. The isolation of the DCCD binding peptide from subunit II, with the goal of comparative sequence studies, forms the major part of the proposed research under this cooperative agreement.
A preliminary sequence (IXLVLTVAVXE) was obtained for a ^{14}C- containing peptide which was isolated from labeled subunit II following cleavage by clostripain and purification on a reverse phase column. There was no similarity to the DCCD binding peptides of F-type ATPases, suggesting that the halobacterial ATPases evolved along different lines than the F-type ATPases.
However, the yield of the identified peptide was very low; thus there may be some ambiguity in the sequence data. In addition, it could not yet be ruled out that multiple labeling of subunit II may have occurred. This would make it difficult to identify the true DCCD binding peptide.
Therefore, a large batch of subunit II was prepared following labeling with ^{14} C-DCCD and electroelution using the established methods. Cleavage of subunit II will be done with several chemical and enzymatic reagents, so as to ascertain binding of DCCD to one or several large fragments. Initial results with cleavage of (unlabeled) subunit II at Met residues by cyanogen bromide indicated a nearly complete reaction.

2. Archaebacteria (or Archaea, according to the new nomenclature) are presumed to have undergone little evolution. This conclusion was reached by C.Woese from 16 S rRNA sequencing experiments, more recently also from 23 S rRNA data. However, there are no other confirmatory data to support this claim. Most interesting with respect to the question of a "slow evolution" are bacterial isolates from salt sediments of great geological age. If it can be established that these bacteria survived since the time when the salts were deposited, they would constitute living systems which should have not been subjected to mutational and selectional events. Vestiges of their evolution may be found in their amino acid and nucleic

acid sequences, particularly those of key enzymes of cellular metabolism, such as the ATPases.
From C. Norton (University of Maine) we had obtained halobacterial strains which were isolated from rock salt of an English salt mine at about 1000 feet depth. Initial characterization of strains 54 R and 54 P of the isolates indicated a resemblance to *H. saccharovorum* on the basis of lipid data (C.Norton, unpublished). We prepared the ATPase from strain 54 R according to our standard protocol. Further characterization of the enzyme was performed at the University of Vienna by graduate student Michael Sulzner and undergraduate Eva Egelseer.
Molecular mass, pH optimum, nitrate sensitivity and subunit structure of the ATPase from strain 54 R were similar as those from the ATPase from *H. saccharovorum* . In addition, membranes from 54 R showed a strong crossreaction of a band of 85 K with the antiserum against subunit A of the vacuolar ATPase (obtained from Dr.E.J.Bowman, University of Santa Cruz), just as had been observed with membranes and purified ATPase from *H. saccharovorum* (Ref.1). However, the content of the acidic amino acids was slightly higher in the ATPase from strain 54 R.
Several additional bacterial isolates from rock salt were obtained from the salt mine near Bad Ischl/Austria. Two strains were compared with known halobacterial strains with respect to morphology, whole cell protein patterns, immunoreactivity and antibiotic sensitivity. One strain, BIr, resembled *H. saccharovorum* , while another isolate (BIp) appeared different and unique. Further characterization of these isolates is in progress.
These results were presented by the P.I. at the XVI. assembly of the European Geophysical Society at a special session "Water in the solar system and its role in exobiology" (Ref.2). It was suggested by the organizers to submit the presentation as a paper for the journal "Origins of Life". This manuscript is now in preparation (Ref.3).

3. During the last working period, labeling of the halobacterial ATPase with the sulfhydryl reagent NEM[2)] suggested the presence of a nucleotide binding site in subunit I, which indicated a further relationship to the vacuolar-type ATPases. The data were presented at a national meeting (Ref.4) and submitted for publication to FEBS Letters. The manuscript was returned since the Editor considered the work preliminary. However, since there are some scattered data from other archaebacterial ATPases implying a similarity to F-type ATPases, we feel that our results could make a

strong point in this respect and therefore will re-submit the manuscript, particularly emphasizing the relationship to vacuolar ATPases (Ref.5).

References

1. Stan-Lotter, H., Bowman, E.J. and Hochstein, L.I. (1991) Relationship of the membrane ATPase from *Halobacterium saccharovorum* to the vacuolar ATPases. Arch.Biochem.Biophys. 284,116-119 .
2. Stan-Lotter,H. (1991) Comparison of proteins from halophilic archaebacteria and viable microbial isolates from ancient salt deposits. Abstr. PS 11, Eur.Geophys.Soc. XVI Ass., April 22-26, Wiesbaden, Germany.
3. Stan-Lotter,H,, Sulzner, M., Egelseer E., Norton, C.F. and Hochstein,L.I. Comparison of proteins from extreme halophiles and microbial isolates from ancient salt deposits (in preparation for "Origins of Life").
4. Sulzner, M., Stan-Lotter, H. and Hochstein, L.I. (1991) Nucleotide-protectable labeling of sulfhydryl groups in subunit I of the ATPase from *Halobacterium saccharovorum* . Abstr.Ann.Meetg. ASM, Dallas, 1991.
5. Sulzner, M., Stan-Lotter, H. and Hochstein, L.I. (1991) Nucleotide-protectable labeling of sulfhydryl groups in subunit I of the ATPase from *Halobacterium saccharovorum* (in preparation).
6. Stan-Lotter, H., Sulzner, M. and Hochstein, L.I. (1991). Functional molecular mass and nucleotide protectable labeling of the membrane ATPase from *Halobacterium saccharovorum* . Gordon Conference,Feb. 1991, Ventura, CA.

Other activities:

February 1991: Participation at the Gordon Conference "Protons and Membrane Reactions", Ventura, CA, and presentation of a poster there (Ref.6).

March - June 1991: Teaching appointment at the University of Vienna, Austria, while remaining at reduced time (25%) with the SETI Institute. Courses taught: Advanced Experimental Microbiology; Archaebacteria.