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ON THE CHIMERICAL NATURE OF THE MEMBRANE-BOUND ATPASE FROM HALOBACTERIUM SACCHAROVORUM

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There is increasing evidence that proton translocating ATPases evolved from a progenitor that had the structural characteristics of vacuolar ATPases (V-ATPases) but, unlike this class of enzymes, was capable of synthesizing ATP. Interestingly, the Archaebacteria, a diverse collection of organisms that diverged from the Prokaryotes at an early stage in the evolution of life, possess ATPases more closely related to V-ATPases than to F_0F_1-ATPases(F-ATPases).

However, an antiserum against the "ß" subunit from S. acidocaldarius reacts with the ß subunit from an F-ATPase as well as subunit II from Halobacterium saccharovorum ATPase (subunit II is probably the functional equivalent to the ß subunit). This communication describes a series of experiments carried out with the goal of determining how the membrane-bound ATPase from H. saccharovorum is related to V- and F-type ATPases, and reflect three approaches: the use of inhibitors; structural studies; and immunological relatedness.

Nitrate is extremely useful for distinguishing between V- and F-ATPases since only the former are inhibited by this reagent. The enzyme from H. saccharovorum is also inhibited by nitrate and at concentrations that inhibit V-ATPases and ATPases from other Archaebacteria. N-ethylmaleimide, which inhibits V- and F-ATPases, also inhibits the ATPase from H. saccharovorum. However, whereas V-type enzymes are inhibited by µM concentrations of this reagent, F-type and the halobacterial enzyme are inhibited by mM concentrations of NEM. 4-chloro-7-nitrobenzofurazan, a nucleotide analog, inhibits V-, F-, and the halobacterial ATPases. In the case of the V-ATPases inhibition is accompanied by the binding of the inhibitor to the largest of the subunits, which is the catalytic subunit. 4-chloro-7-nitrobenzofurazan binds to the ß-subunit of F-ATPases, which is the catalytic subunit. 4-chloro-7-nitrobenzofurazan binds to subunit II of the halobacterial enzyme, which also contains the dicylcohexylcarbodiimide-binding site. In spite of this apparent similarity, the halobacterial enzyme is unrelated to F-type ATPases based on a comparison of the isoelectric points, the amino acid compositions, the molecular mass, and the proteolytic products of the two largest subunits from each enzyme. Furthermore, antisera prepared against the largest subunit from the V-ATPase from Neurospora crassa reacts with subunit I from the halobacterial enzyme. On the basis of these observations, the halobacterial enzyme may represent a chimera. Subunit II appears to be immunologically and functionally related to the ß subunit from F-ATPases. On the other hand, subunit I may be structurally related to the largest of the largest subunits from V-ATPases but possibly functionally related to the α-subunit from F-ATPases (which has a regulatory function). Whether this reflects the nature of the progenitor enzyme or mixture of structure and function which arose after the evolution of the proton ATPases into V- and F-type enzymes is not clear, but it is another example of the evolutionary richness of the extremely halophilic bacteria.