Active transport of Ca\(^{2+}\) in bacteria: bioenergetics and function

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Summary

The bioenergetics of Ca\(^{2+}\) transport in bacteria are discussed with special emphasis on the interrelationship between transport and other cellular functions such as substrate oxidation by the respiratory chain and oxidative phosphorylation. The unusual polarity of Ca\(^{2+}\) movement provides an exceptional tool to compare active transport and other ATP requiring or generating processes since this ion is actively taken up by everted vesicles in which the coupling-factor ATPase is exposed to the external medium. As inferred from studies with everted vesicles, the active extrusion of Ca\(^{2+}\) by whole cells can be accomplished by substrate driven respiration, hydrolysis of ATP or as in the case of *Streptococcus faecalis* by a nonhydrolytic unknown process which involves ATP directly. Substrate oxidation and the hydrolysis of ATP result in the generation of a pH gradient which can energize the Ca\(^{2+}\) uptake directly (Ca\(^{2+}\)/H\(^+\) antiport) or via a secondary Na\(^+\) gradient (Ca\(^{2+}\)/Na\(^+\) antiport). In contrast to exponentially growing cells sporulating Bacilli accumulate Ca\(^{2+}\) during the synthesis of dipicolinic acid. Studies involving Ca\(^{2+}\) transport provided evidence in support of the hypothesis that the Mg\(^{2+}\) ATPase from *Escherichia coli* not only provides the driving force for various cellular functions but exerts a regulatory role by controlling the permeability of the membrane to protons. The different specificity requirements of various naphthoquinone analogs in the restoration of transport or oxidative phosphorylation, after the natural menaquinone has been destroyed by irradiation, has indicated that a protonmotive force is sufficient to drive active transport. However, in addition to the driving force (protonmotive force) necessary to establish oxidative phosphorylation, a specific spatial orientation of the respiratory components, such as the naphthoquinones, is essential for the utilization of the proton gradient or membrane potential or both. Finally, evidence suggesting that intracellular Ca\(^{2+}\) levels might play a fundamental role in bacterial homeostasis is discussed, in particular the role of Ca\(^{2+}\) in the process of chemiotaxis and in conferring bacteria heat stability. A vitamin K-dependent carboxylation reaction has been found in *Escherichia coli* which is similar to that reported in mammalian systems which results in \(\gamma\)-carboxylation of glutamate residues. Although all of the proteins containing \(\gamma\)-carboxyglutamate described so far are involved in Ca\(^{2+}\) metabolism, the role of these proteins in *Escherichia coli* is unknown and remains to be elucidated.

Abbreviations used

FCCP, carbonylcyanide p-trifluoromethoxyphenylhydrazone; DCCD, N,N'dicyclohexylcarbodiimide; CCCP, carbonylcyanide m-chlorophenylhydrazone; PMS, phenazine methosulfate; D-LDH, D-lactate dehydrogenase; TPD, N,N,NN'-tetramethyl-p-phenylenediamine; pCMPS, p-chloromercuriphenylsulfonate; HQNO, 2n-heptyl-4-hydroxyquinoline-N-oxide; EGTA, ethyleneglycol-bis(amo-no-ethyl-ether)-N,N'-tetraacetic acid; TCS, tetrachlorosalicylanilide; pCMBS, p-chloromercuribenzenesulfonic acid; pCMB, p-chloromercuribenzoate, \(\Delta\mu_{H^+}\), protonmotive force; \(\Delta\psi\), membrane potential; \(\Delta p\), transmembrane proton gradient.

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I. Introduction

A number of bacterial systems have been used to study membrane associated phenomena, such as the sequence of electron transport, oxidative phosphorylation and active transport of solutes. Membranes of bacterial origin constitute an ideal model system for studying these membrane associated processes since components such as electron transport carriers, binding or carrier proteins for solute translocation and coupling factor ATPase can be studied in a homogeneous population of whole cells (wild type and specific mutants) or with different types of intact membrane structures derived from whole cells, which differ in size and in vectorial orientation. Thus, a meaningful comparison of the requirements and insight concerning the mechanism of energy transduction for active transport and oxidative phosphorylation can be gained from studies with the different types of membrane structures.

Two excellent reviews on calcium transport in microorganisms have been written by Silver within the past 3 years (1, 2). Therefore, it is not the intent of the present review to be redundant or discuss all the information available on active transport of calcium in bacterial cells, but instead to present the pertinent studies which have lead to the development of our present understanding of the bioenergetic mechanisms involved in the active transport of calcium ions and to provide a comparison to other membrane related energy transducing systems. In addition, an attempt will be made to provide some insight as to the role of calcium in cellular metabolism.

Although calcium is found in bacterial cells its distribution across the cell membrane is unusual since the internal concentration of this ion is low when compared to the external environment. Evidence has been presented that bacteria contain a specific and active process for maintaining low levels of calcium ions within the cytoplasm (1, 2). Whole cells and protoplasmic ghosts exclude Ca\(^{2+}\) whereas everted membrane vesicles take up Ca\(^{2+}\) against a concentration gradient. In essence, the regulation of calcium ions in bacterial cells is accomplished by a specific efflux of this ion, in certain instances in exchange for other essential ions. In contrast to exponentially growing cells sporulating Bacilli have been shown to accumulate Ca\(^{2+}\) concomitant with synthesis of dipicolinic acid (3, 4). High Ca\(^{2+}\) levels have also been found to be associated with vegetative termophylic growth (5).

Calcium uptake in everted membrane vesicles is accomplished by substrate driven respiration, hydrolysis of ATP (1, 2) or in certain microorganisms by a nonhydrolytic unknown process which involves ATP directly (6). Substrate oxidation and the hydrolysis of ATP result in the generation of a pH gradient which can energize the Ca\(^{2+}\) uptake directly (Ca/H\(^{+}\) antiport) (7, 8, 9, 10) or via a secondary Na\(^{+}\) gradient (Ca\(^{2+}\)/Na\(^{+}\) antiport) (11).

The role of Ca\(^{2+}\) in bacteria is not clear at the present time. It has been proposed that Ca\(^{2+}\) might play a regulatory role by maintaining exoenzymes in an inactive form until the time that they are secreted from bacterial cells since Ca\(^{2+}\) specific enzymes are always extracellular or membrane bound (1). Recently it has been suggested that internal Ca\(^{2+}\) levels might play a fundamental role in the mechanism of chemiotaxis, one of the most primitive sensory-motor responses (12). Proteins of mammalian origin containing \(\gamma\)-carboxyglutamic acid residues have been shown to be involved in calcium-binding (13). The presence of \(\gamma\)-carboxyglutamatic acid residues has also been shown in certain proteins associated with cytosol and ribosomal fraction from *Escherichia coli* under conditions of rapid growth, but the nature and role of these proteins remains unknown (14).

Since the process of active transport is inextricably associated with other membrane related phenomena, such as respiration and oxidative phosphorylation, an effort will be made to discuss the active transport of Ca\(^{2+}\) in relation to these other membrane functions. In particular, attention will be drawn to the role of naphthoquinones as essential components of the energy transducing apparatus involved in active transport and oxidative phosphorylation (15, 16).

Oxidative phosphorylation in bacteria was demonstrated about 15 years following the discovery of this process in mitochondria. Hersey and Ajl (17), and Pinchot and Racker (18) first reported a coupling process in *E. coli* whereas Pinchot described a similar process in *Alcaligenes faecalis* (19) and Brodie and Gray in *M. phlei* (20, 15). The reaction first described for the *E. coli* system (18), however, failed to eliminate the