Symposia and Colloquia Lectures

ANAEROBIC ELECTRON TRANSPORT IN BACTERIAL ENERGY CONVERSION

A.H. Stouthamer
Biological Laboratory, Vrije Universiteit, Postbus 7161,
1007 MC Amsterdam, The Netherlands

Bacteria can utilize a large variety of compounds as terminal electron acceptor instead of oxygen, e.g. oxidized nitrogen compounds. The first step in nitrate dissimilation is a reduction to nitrite. Two ways of dissimilatory nitrite reduction can be distinguished. Nitrite can be converted to gaseous products (nitrous oxide or nitrogen) or to ammonia. The ATP gain during these reductions is dependent on: 1. the respiratory chain for electron transport towards these nitrogenous oxides. 2. the side of the membrane at which the reduction takes place. 3. the number of proton pumps present in the respiratory chain of the organism. Generally cytochrome b is the electron donor for the reduction of nitrate and cytochrome c the electron donor for the reduction of nitrite (to nitrous oxide or ammonia) and of nitrous oxide. The reduction of nitrate always occurs at the cytoplasmic side of the membrane. However nitrite and nitrous oxide are reduced at the periplasmic side of the membrane. By a reduction at the periplasmic side of the membrane the charge separation is reduced. Oxidation of some substrates may occur at the periplasmic side of the membrane, by which the charge separation is increased. Electron transfer and proton translocation are shown in Fig. 1 for Paracoccus denitrificans and Campylobacter sputorum. The site 2 region in the electron transport chain in P. denitrificans has been split in two proton-translocating segments. During electron transport to nitrate site I and IIa and during electron transport to oxygen, nitrite and nitrous oxide site I, IIa and IIB are passed. After aerobic growth sometimes also site III is present. In Escherichia coli the $\rightarrow H^+/O$ and $\rightarrow H^+/NO_3^-$ ratios are 4 and measurements of molar growth yields indicate that the stoichiometry of ATP formation is the same with oxygen and nitrate. E. coli contains site I and IIa which function in electron transport to oxygen and nitrate. In C. sputorum only site IIa is present. The charge separation during electron transport in these organisms is shown in Table 1. The results are in accordance with calculations of the stoichiometric ATP gain from molar growth yields using a $\rightarrow H^+/ATP = 3$. These findings can not be explained easily by a Q cycle model for electron transfer and energy generation. For this purpose a b cycle model seems more appropriate.

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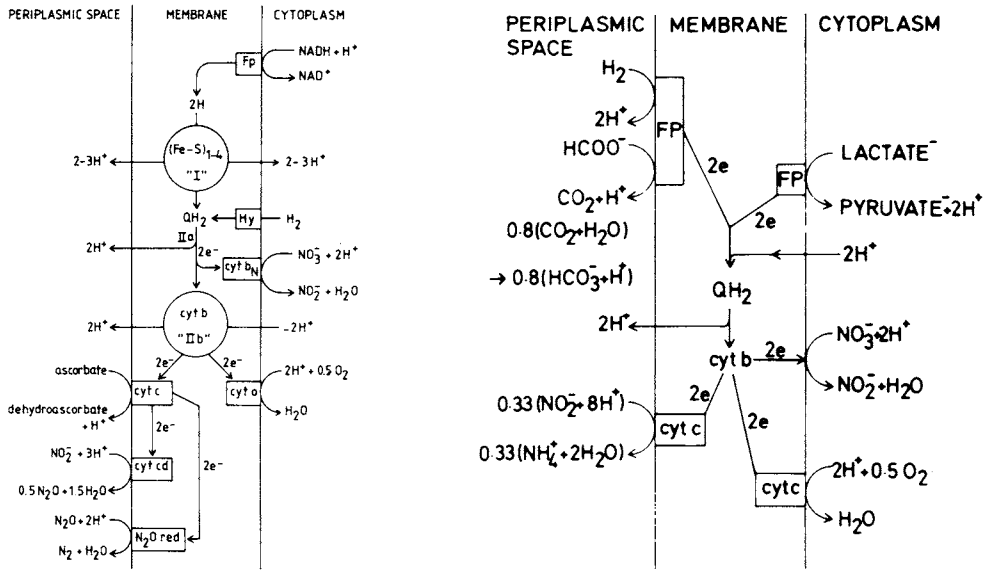

Fig. 1. Simplified scheme for proton translocation and electron transport in P. denitrificans (left) and C. sputorum (right). Fp, flavoprotein, QH_2, ubiquinol; Q, quinone; Hy, hydrogenase; Fe-S, iron-sulphur center.

Table 1. Number of charges translocated across the cytoplasmic membrane during flow of $2e^-$ ($\rightarrow q^+/2e^-$ ratio) from various substrates to an electron acceptor in P. denitrificans, E. coli and C. sputorum.

Electron acceptor reaction		P. denitrificans NADH	P. denitrificans H_2 or succinate	E. coli NADH or formate	E. coli lactate	C. sputorum lactate	C. sputorum H_2 or formate
		$\rightarrow q^+/2e^-$					
O_2	→ H_2O	7	4	4	2	2	4
O_2	→ H_2O (cyt aa_3)	9	6	–	–	–	–
NO_3^-	→ NO_2^-	5	2	4	2	2	4
NO_2^-	→ $\frac{1}{2}N_2O$	5	2	–	–	–	–
N_2O	→ N_2	5	2	–	–	–	–
NO_2^-	→ NH_4^+	–	–	–	–	0	2

METHANOGENESIS

R. Thauer
Mikrobiologie, FB Biologie, Philipps-Universität
Marburg, D-3550 Marburg/Lahn (FRG)

Methanogenic bacteria ferment H_2 and CO_2, formate, methanol, methylamines and/or acetate to methane. Per mol methane 0.5 to 1.0 mol ATP is generated. Coupling is propably via the chemiosmotic mechanism (1). Sodium ions are somehow involved (2). The exergonic step of methanogenesis is catalyzed by methyl-CoM reductase which contains a nickel porphinoid as prosthetic group (3-6).

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BIOENERGETIC PROBLEMS OF ALKALOPHILIC BACTERIA

T.A. Krulwich and A.A. Guffanti
Department of Biochemistry, Mount Sinai School of Medicine
of the City University of New York,
New York, New York 10029 USA

Alkalophiles live at external pH values of 10.5 and higher, but maintain a cytoplasmic pH at $\leq$ pH 9.5. While the $\Delta\psi$, positive out, is substantial, the total $\Delta\bar{\mu}_{H^+}$ is low at optimal pH values because of the large "reversed" ΔpH [1].

Several lines of evidence indicate a crucial role for Na^+ in pH homeostasis [2]. Na^+ may function via an electrogenic Na^+/H^+ antiporter in respiring cells. The antiporter is inhibited by $[H^+]_{in}$ and is, accordingly, relatively inactive at neutral pH. Indeed, pH homeostasis in the neutral pH range may be a problem for most alkalophiles. In highly Na^+-dependent alkalophiles, e.g., B. firmus RAB, Na^+/H^+ antiport activity may have an exclusive role in "acidifying" the interior. Na^+ circulation is completed by a plethora of Na^+/solute symporters. Na^+ is the major coupling ion for transport. The K_m for Na^+-dependent symporters correlates with that of the Na^+/H^+ antiporter in a given species. In less Na^+-dependent species, e.g., B. alcalophilus, there may be an auxiliary role for a K^+/H^+ antiporter.

The membranes of alkalophilic bacilli contain enormous quantities of respiratory chain components, especially large amounts of b- and c-type cytochromes. There are several b-species, a single c-cytochrome, and cytochromes aa3, as determined by midpoint potentials. A Rieske Fe-S protein, at the same potential as the cytochrome c, is present in B. alcalophilus [3,4]. Cytochrome oxidase purified from B. firmus RAB contains subunits of 56K and 40K as well as a mole of bound cytochrome c. Cells of B. firmus RAB, using endogenous substrates exhibit high H^+/O ratios at pH 9.0. The H^+/O ratios are much lower at neutral pH [5]. Observations of structural/functional features of the alkalophile respiratory chain suggest that this chain is especially adapted to function well at high pH.

ATP synthesis via oxidative phosphorylation occurs at low $\Delta\bar{\mu}_{H^+}$ values in cells and in ADP + P_i-loaded vesicles. No requirement for or stimulation by Na^+ is found [1]. Rather, an H^+-translocating F_1F_0-ATPase appears to function at low $\Delta\bar{\mu}_{H^+}$ values, as long as that $\Delta\bar{\mu}_{H^+}$ is derived from respiration. Artificial $\Delta\psi$ values of comparable magnitudes to those formed by respiration do not energize ATP synthesis at pH 9.0. Even at pH 7.0, respiration is more efficacious than a comparable, valinomycin-induced K^+ diffusion $\Delta\psi$ in energizing ATP synthesis [6]. A localized pathway for H^+ movements between pumps and sinks may be particularly crucial for alkalophiles and, hence, particularly amenable to study (e.g., genetically) in these organisms.

Current studies are directed at genetic as well as biochemical approaches to the interesting questions of alkalophile bioenergetics. It has been possible, thus far, to transform B. firmus RAB to antibiotic resistance using plasmids from Bacillus subtilis.

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SODIUM ION TRANSPORT AS PRIMARY ENERGY SOURCE FOR ATP SYNTHESIS

P. Dimroth and W. Hilpert
Institut für Physiologische Chemie der TU München
Biedersteiner Straße 29, 8000 München 40, FRG.

In certain anaerobic bacteria, Na^+ transport is driven by decarboxylation reactions [1]. These decarboxylations are indispensible steps of the respective fermentation pathways and proceed by a large negative free energy change (6-7 kcal/mol) which provides the driving force for the transport. In this way, part of the decarboxylation energy is conserved and can be used by the cell to drive endergonic membrane reactions. Three different enzymes have been recognized to function as sodium transport decarboxylases. These are oxaloacetate decarboxylase from Klebsiella aerogenes [2], methylmalonyl-CoA decarboxylase from Veillonella alcalescens [3] and glutaconyl-CoA decarboxylase from Acidaminococcus fermentans [4].

Growing cells of V. alcalescens maintain a Na^+ concentration gradient of about 1:10 from inside to outside, probably due to the action of methylmalonyl-CoA decarboxylase. The usefulness of this Na^+ gradient as a driving force for endergonic chemical synthesis has been demonstrated by the reversion of malonyl-CoA decarboxylation with a reconstituted proteoliposomal system. Malonyl-CoA was formed from acetyl-CoA and CO_2 when a Na^+ concentration gradient inside > outside was applied but not after dissipating the Na^+ gradient with monensin. The Na^+ gradient could either be imposed by diluting Na^+-loaded vesicles into a Na^+-free buffer or by the Na^+ pump oxaloacetate decarboxylase with proteoliposomes containing oxaloacetate decarboxylase in addition to methylmalonyl-CoA decarboxylase. The latter system is infact a transcarboxylase in catalyzing the formation of pyruvate and malonyl-CoA from oxaloacetate and acetyl-CoA and vice versa. This transcarboxylation strictly depends upon the circulation of Na^+ ions and is thus completely different from the classical transcarboxylase from Propionibacterium shermanii. With the reconstituted proteoliposomal systems decarboxylation and Na^+ uptake were tightly coupled, so that per decarboxylation of 1 mol oxaloacetate or malonyl-CoA 2 mol Na^+ ions were translocated through the membrane.

In certain organisms, a decarboxylation derived Na^+ gradient is the only source of energy to support ATP synthesis. As an example, Propionigenium modestum, a strictly anaerobic bacterium, grows from the fermentation of succinate to propionate and Co_2 [5]. Intermediates of the fermentation pathway are succinyl-CoA and methylmalonyl-CoA. Decarboxylation of methylmalonyl-CoA to propionyl-CoA proceeds by generation of a Na^+ gradient which is the only biologically useful energy gained in this fermentation process. This Na^+ gradient in turn drives ATP synthesis via a Na^+ stimulated ATPase which is present in high amounts in the bacterial membrane. These decarboxylation-dependent energy conservations are distinct from all other membrane-linked energy conservation mechanisms since no electron transport chains are involved and since Na^+ and not H^+ is functioning as the coupling ion.

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THE REGULATION OF SOLUTE TRANSPORT IN BACTERIA

W.N. Konings, M.G.L. Elferink, K.J. Hellingwerf, B. Poolman, J.M. van Dijl
Dept. of Microbiology, University of Groningen, Kerklaan 30, 9751 NN HAREN, The Netherlands

Solute transport across the cytoplasmic membrane of bacteria can occur via several mechanisms: the primary transport systems, which convert chemical or light energy into electrochemical energy; the secondary transport systems which convert one form of electrochemical energy into another form and the group translocation systems which modify the solute chemically during the translocation process (1). The primary transport systems comprise the systems which generate an electrochemical gradient of protons (or sodium) such as the membrane-bound proton translocating ATPase and the cytochrome-linked electron transfer systems. The secondary transport systems are driven by the electrochemical gradients.

The proton motive force or its components, the membrane potential and the pH-gradient across the cytoplasmic membrane has been demonstrated to be the driving force for the uptake of many solutes in bacteria (1). Several solutes are translocated by proton-solute symport systems but the number of protons and charges translocated with the solute can vary (2). This variable proton-solute stoichiometry has been studied extensively for lactate transport in Escherichia coli (3,4) and Streptococcus cremoris (5,6). These results have supplied experimental support for the Energy Recycling Model which postulates that the efflux of endproducts in fermentative bacteria leads to the generation of a proton motive force and supplies metabolic energy to the organisms (7).

Recent studies in Rhodopseudomonas sphaeroides (8,9) and E. coli (10) have shown that the rate of secondary transport processes is determined by the proton motive force but in addition independently of the proton motive force by the rate of electron transfer in the electron transfer systems: the cyclic electron transfer system in Rps. sphaeroides grown under anaerobic conditions in the light and the respiratory chain in E. coli and Rps. sphaeroides grown under aerobic conditions in the dark. Kinetic analysis of solute uptake indicates that the activity of the electron transfer systems determines the fraction of active transport carrier molecules in the cytoplasmic membrane (9). A similar regulation by the rate of electron transfer has been reported for photophosphorylation in Rps. capsulata (11) and Rps. sphaeroides (12).

The regulation by electron transfer is exerted on proton solute-

and on sodium-solute symport systems which argues against the involvement of localized chemiosmotic phenomena in this process.

Studies in E. coli have shown that the membrane-bound protein Enzyme II of the PEP-dependent sugar group translocation system (13,14) and the carrier proteins of lactose and proline (15,16) contain redox-active disulphide groups. The redox state of these groups is determined by the redox potential of the environment and by the proton motive force. It is very likely that the activity of these proteins is also controlled by a redox interaction with (a) components(s) of the electron transfer system and that this direct interaction can explain the regulation of the transport activity by the activity of the electron transfer systems.

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